IMPERIAL INSTITUTE

OF

AGRICULTURAL RESEARCH, PUSA.

東北帝國大學

理科報告

(生 物 學)

第四卷

THE

SCIENCE REPORTS

OF THE

TÔHOKU IMPERIAL UNIVERSITY

FOURTH SERIES

(Biology)

SENDAI, JAPAN

Vol. IV.

CONTENTS

No. 1, Fasc. 1.

(Published February, 15th, 1929)

No. 1, Fasc. 2.

(Published April 25th, 1929)

No. 2.

(Published July 10th, 1929)

No. 3.

(Published September 28th, 1929)

No. 4.

(Published December 13th, 1929).

Rhizoid Formation in the Embryo of *Turbinaria* (?) *fusiformis* Yendo and *Sargassum Thunbergii* O'Kuntze.

By

Masato Tahara.

As emphasized in my previous paper on *Coccophora Langsdorfii* (Tahara, 1928), it seems to me that the study of the rhizoid formation in the embryo of Fucaceous algae gives us an important clue for the determination of the systematic position of each species. For the verification of this opinion it is, however, necessary to investigate the rhizoid formation in a number of algae belonging to this family. In the present paper rhizoid formation of only two species, *Turbinaria* (?) *fusiformis* Yendo and *Sargassum Thunbergii* O'Kuntze will be treated. The same study on other species of Fucaceae will be published in a series of subsequent papers.

1. TURBINARIA (?) FUSIFORMIS YENDO.

This species is one of the commonest algae of Japan. The reproduction begins at the beginning of June and continues till July. The plants are strictly dioecious. The liberation of sexual cells occurs simultaneously and periodically, as is usual in other members of Fucaceae, and the discharged eggs remain attached to the receptacle for two or three days. Eggs contained in one receptacle are not generally liberated all together, but at two or three different times. Sperms are biflagellate and have no eye-spot.

The eggs at the moment of liberation have eight nuclei and appear to be fertilized in this stage. The first segmentation wall runs perpendicular to the long axis of the embryo. A lens-shaped rhizoid cell, the intial cell for rhizoid formation, is cut off, as a rule, by the second segmentation wall. The first two division walls in this rhizoid cell run perpendicular to each other, as a result forming four equal-shaped quadrant cells. The third division walls, however, run in

various directions, some of them being parallel to the former ones, others oblique without passing through the center of the rhizoid cell and still others radial, passing through the center of the cell (Fig. 1).
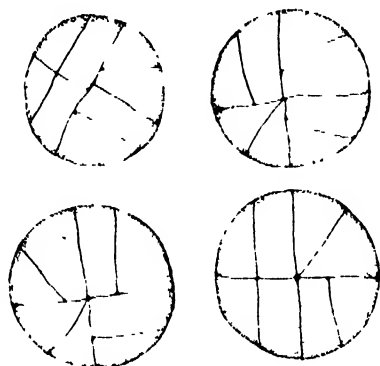
Text-fig 1.   Eight cell stage of the rhizoid cell of *Turbinaria* (?) *fusiformis*.
(×420)

Rhizoid formation begins in this eight cell stage of the rhizoid cell. One rhizoid being produced in each cell, a group of eight rhizoids is developed at one extremity of the embryo (Fig. 2). Rhizoids which have no direct relation to the rhizoid cell can not be seen, at least in the early stage of development. Thus the mode of rhizoid formation of this alga is seen to have a deep resemblance to that of *Coccophora Langsdorfii*. But the frequent occurence of oblique and radial division-walls in the rhizoid cell of *Turbinaria* (?) *Thunbergii* appears to throw out a hint of a relationship to *Sargassum*.

As to the systematic position of this alga, at present no definite opinion yet prevails. The plant was first described by HARVEY from sterile specimens under the name, *Cystophyllum fusiforme*. Later YENDO, after finding out that the receptacles were borne in an axillary position, removed the plant from the Genus *Cystophyllum* and provisionally suggested the name *Turbinaria* (?) *fusiformis*. So far as I am aware, there is no publication concerning the rhizoid formation in the species *Turbinaria*. So for the present it is impossible to compare the result here obtained to that of any other study on *Turbinaria*. But it seems to me certain that this plant should be placed under a genus other than *Sargassum*, because the rhizoid

formation of this plant greatly differs from that observed in the species of *Sargassum* (TAHARA. 1913, 1928).
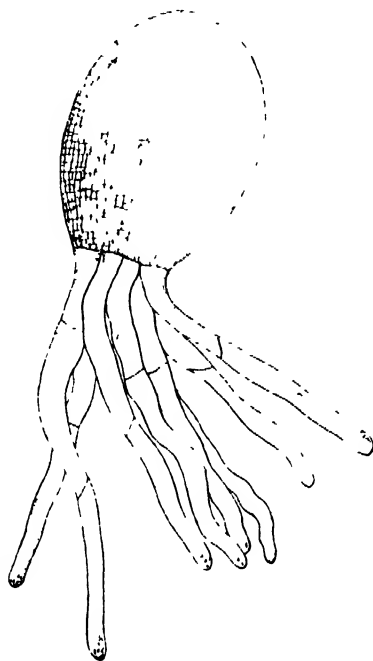
Text-fig. 2.   Embryo of *Turbinaria* (?) *fusiformis*.   ($\times 160$)

The present study was carried out at the Misaki Marine Biological Station of the Tokyo Imperial University. I wish to express here my hearty thanks to Prof. N. YATSU, and Dr. M. OSHIMA of the Station.

2. SARGASSUM THUNBERGII O'KUNTZE.

In his preliminary list of Japanese Fucaceae, YENDO gives the name *Turbinaria Thunbergii* to the plant under consideration. But in a full report published later he has changed his opinion and transferred the plant into the genus *Sargassum*. In any case, this plant has a certain characteristic, not common in *Sargassum*. Several forms, differing much in their appearance, are known in the present species. Fulcrant leaves in one form are mostly transformed into

vesicles, especially in its younger stage of development, thus suggesting
a close affinity of the plant to *Turbinaria*(?) *fusiformis*.

The male and female receptacles of the plants, which are also
strictly dioecious, ripen in July and August in the vicinity of the
Asamushi Biological Station, where the material of the present inves-
tigation was obtained by Mr. M. SHIMAKURA at my request. According
to my observation of several years, the liberation of sexual cells of
this plant appears to occur always on a day of neap tide. This
circumstance may be quite favorable to the plants, as they grow
generally at the high tide mark, and on the days of spring tide most
of them lie naked for several hours, exposed miserably to the direct
sunshine. If the liberation of sexual elements occured on a day of
spring tide, they would probably be easily destroyed by the heat of
the summer sun.

Turbinaria(?) *fusiformis* grows also at the high tide mark. One
general liberation of sexual cells of this plant occured in Misaki this
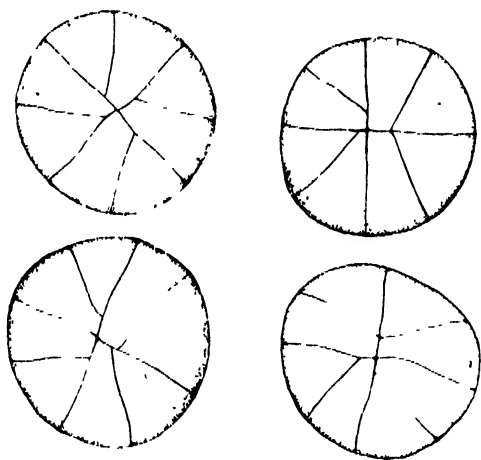
Text-fig. 3. Eight cell stage of the rhizoid cell of *Sargassum Thunbergii*.
(× 420)

year on July 11, a day of neap tide. It seems to me quite probable
that the sexual cells of this plant are also always discharged on a
day of neap tide, although at present no extensive data as to this
point are available.

Early stages of development in *Sargassum Thunbergii* are about the same as those in other species of *Sargassum*, but the mode of
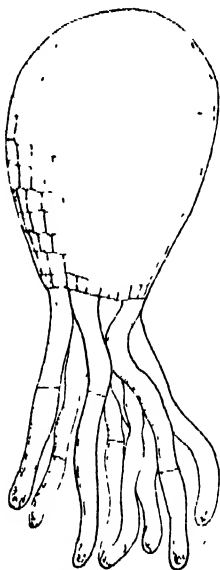
Text-fig. 4. Embryo of *Sargassum Thunbergii*. (×210)

division in the rhizoid cell differs distinctly from the usual course in *Sargassum* and, much resembles that of *Turbinaria* (?) *fusiformis*. The only difference is that in *Sargassum Thunbergii* the third division-walls in the rhizoid cell, without passing through the center, run mostly oblique to the former ones (Fig. 3). As is shown in Text-fig. 4, eight rhizoids are developed at one extremity of the embryo; rhizoids not originating from the rhizoid cell are not seen, at least in the early state of embryonal development. On my opinion therefore, this species ought to be placed under the same genus with *Turbinaria* (?) *fusiformis*.

In conclusion, the author should like to express his obligation to Mr. M. SHIMAKURA, who has helped him much in the present investigation. He also wishes to acknowledge his indebtness to " The Saito Gratitude Foundation " for financial aid.

6   MASATO TAHARA

LITERATURE CITED.

TAHARA. M. 1913. Oogonium liberation and the embryogeny of some Fucaceous algae.
    Journ. Coll. Sc. Imp. Univ. Tokyo, Vol. 32, Art. 9, pp. 1–13.
TAHARA. M. 1928. Contributions to Morphology of *Coccophora Langsdorfii* (TURN.)
    GREV. Sc. Rep., Ser. 4. Biology, Tôhoku Imp. Univ., Sendai, Japan, Vol.
    31, No. 4, Fasc 2. pp. 727–732.
YENDO. K. 1907. The Fucaceae of Japan. Journ. Coll. Sc. Imp. Univ. Tokyo, Vol.
    21, Art. 12, pp. 1–174.

A New Terrestrial Amphipod *Orchestia kokuboi*, sp. nov. from Asamushi.

By

Masuzô Uéno.

(Kyôto Imperial University)

Through the courtesy of Professor T. Komai, 1 recently obtained four specimens of a terrestrial amphipod which had been collected by Professor S. Kokubo of the Asamushi Marine Biological Station. A careful examination of these crustaceans has revealed that they have not yet been described. A short description of this new species, therefore, is given below.

Orchestia kokuboi, sp. nov.

Body laterally compressed, whitish with a pink tint. Eyes rather large, nearly round, blackish, situated near the antero-dorsal corners of head. Antennule (Fig. 1, A) extending far beyond the end of the penultimate joint of peduncle of antenna, but scarcely reaching the end of third joint; flagellum 6–7 jointed, about twice as long as third joint of peduncle. Antenna (Fig. 1, A) rather slender, much less than half as long as body; flagellum a little longer than peduncle, 19-20 jointed. Mouth-parts prominently projecting below; endpod of maxillula vestigial, lobe of first joint slender and with two apical setæ (Fig. 1, B); lobe of second joint of maxilla with a large seta on apical end (Fig. 1, C); maxillipeds, palpal joints short and broad, fourth joint entirely wanting (Fig. 1, D). First gnathopod in male subchelate; fourth joint without apical process; fifth somewhat dilated distally, spinulose, with a prominent subapical process; sixth with a large apical lateral expansion, spinulose, with finger fitted closely to apical margin (Fig. 1, E). Second gnathopod in male distinctly chelate; second joint long, parallel-sided, not widened distally; sixth very large and broad, oval, apical margin spinose; finger or claw large, curved, with distal extremity blunt, and inner margin fitted closely to oblique
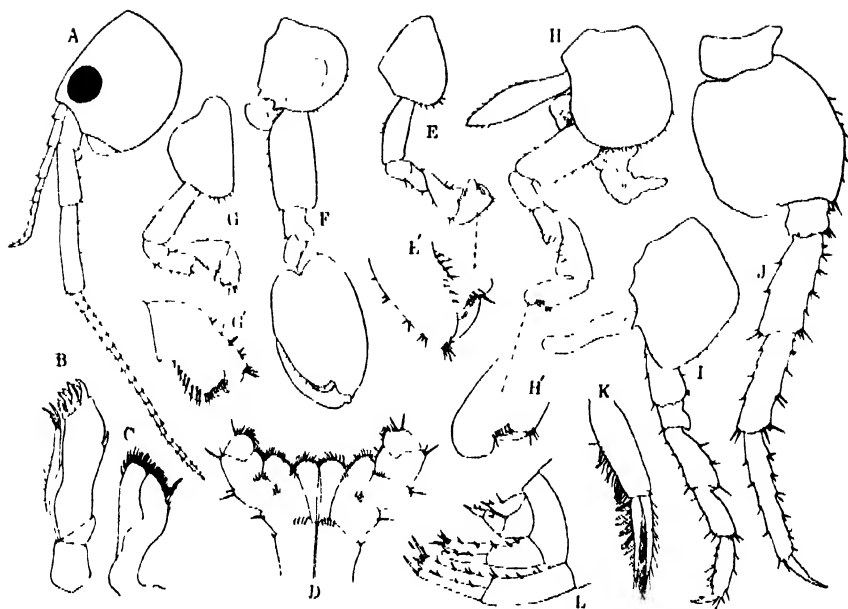
Text-figure 1.

Orchestia kokuboi sp. nov.

A. Head, antennule and antenna of male, lateral view, ×8; B. Maxillula, ×20;
C. Maxilla, ×20; D. Maxillipeds, ×20; E. Right 1st gnathopod of male,
×8, E′ ×20; F. Right 2nd gnathopod of male, ×8; G. Right 1st gnathopod
of female, ×8, G′ ×20; H. Right 2nd gnathopod of female, ×8, H′ ×20;
I. Right 2nd peraeopod of male, ×8; J. Right 5th peraeopod of female, ×8;
K. Right 3rd pleopod of male, ×12; L. Uropods and telson, lateral view, ×8.

convex apical margin of sixth joint (Fig. 1, F). First gnathopod in
female subchelate; fifth and sixth joints spinulose, the latter compara-
tively small, parallel-sided; finger nearly as long as entire apical margin
of sixth joint (Fig. 1, G). Second gnathopod in female subchelate;
second joint somewhat lamelliform, front and hind margins nearly
straight; fifth joint distally widened, spinulose; sixth long, apical
margin with a large round process produced beyond finger (Fig. 1, H).
Second peraeopod, inner margin of finger with a spine (Fig. 1, I).
Of fourth and fifth peraeopods, fourth, fifth and sixth joints very
long, spinose, finger slender and acutely pointed (Fig. 1, J). Third
pleopod normal (Fig. 1, K). Third pleon segment, postero-lateral

corners quadrate, angle somewhat acute but not produced. First uropod, rami equal in length and a little shorter than peduncle, with margins spinose ; rami of both second and third uropods also a little shorter than their peduncles (Fig. 1, L) ; telson small, cone-shaped and spinulose.

Length of body 18 mm. in male, 17 mm. in female (exclusive of antennæ).

Locality : Four females and a male were collected by Professor S. KOKUBO on December 8, 1927, on a hill in Yu-no-shima, a small island off Asamushi (latitude about 40°70′N). This spot is far above the tide-marks ; and the animals were found living in burrows in soft damp soil under dead leaves.

Remarks : This new species closely resembles the North American species *Orchestia grillus* (BOSC, 1802)[1], but shows 'differences in the structures of the antennules, gnathopods and in other features. The antennule of this species almost reaches the end of the third joint of the peduncle of the antenna, while in *O. grillus* it does not come beyond the penultimate joint of the peduncle of the antenna. The second joint of the second gnathopod in the female of this new species is parallel-sided, but in *O. grillus* the front margin is strongly convex except at the distal end. The sixth joint of the second gnathopod in the male of this new species is slightly narrower towards the palm, while in *O. grillus* it has nearly the same width throughout. These characters of the gnathopods of the present species remind one rather of *Orchestia humicola*, a terrestrial amphipod described by EDWARD VON MARTENS[2] from Japan.[2] *O. humicola*, however, is a smaller species only 8 mm. long, which differs from the present species in the following features : (i) the flagellum of the antennule is 3-5-jointed ; (ii) the second joint of the fifth peraeopod is strongly serrate or crenulate on the upper part of the hind margin ; (iii) the rami of the first uropod are considerably shorter than the peduncle.

<div align="right">Kyôto, October 25, 1928.</div>

[1] STEBBING, T. R. R. — "Amphipoda-Gammaridea" in. SCHULZE ᴺ Tierreich, Lief. 21, 1906, p. 540.

[2] MARTENS, E. v. — „Über einige ostasiatische Süsswasserthere", Archiv f. Naturges., Jahrg. 34, Bd. 1, pp. 56–57. 1868.
The locality is not recorded.

Report of the Biological Survey of Mutsu Bay.
12.   Cheilostomatous Bryozoa of Mutsu Bay.*

By

Yaichirô Okada.
(With Plates I–V.)

This is the second paper on the Bryozoa of Mutsu Bay, dealing with the Cheilostomata. Twenty-seven species are recorded, six of which seem to be new to science. The observations herein given confirm the zoogeographical conclusions mentioned in the preceding paper. It is interesting to note that the Reteporan species is limited to *Retepora terebrata* Buchner, though this species is very abundant. This is the first time it has been observed that different kinds of substratum cause different outer configurations in *Costazia costazi* Audouin, when the zooecial features are entirely similar.

Order CHEILOSTOMATA Busk.
Suborder **ANASCA** Levinsen.
Division I.   MALACOSTEGA Levinsen 1909.
Family 1.   **BIFLUSTRIDAE** Smitt 1872.
Genus 1.   CONOPEUM Gray 1848.

1.   Conopeum serrata (Hincks).
(Text-fig. 1.)

Membranipora membranacea form *serrata* Hincks, 1882, A. M. N H., vol. 10, p. 469.

Membranipora seriata Robertson, 1908, Univ. Calif. Pub. Zool, vol. 4, pp. 268–269, pl. 16, figs. 20, 21, 21 a.

Diagnosis: Zoaria consisting of circular or lobate patches incrusting seaweed. Zooecia quadrangular, oblong, alternate, arranged in lines radiating from the centre of the colony; aperture occupying the whole of the front, closed by a membranous front wall; margins

---

*A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

raised, with a crenated, inner rim, some of the crenations growing long and forming distinct denticles, and with a short spine at each distal angle; operculum curved, its margin strengthened by a chitinous rib. Avicularia and ooecia are wanting.

Several colonies were obtained off Ishihama (Station 43; Sp. no. 617). They were attached to Ulva and are light greyish in alcohol.
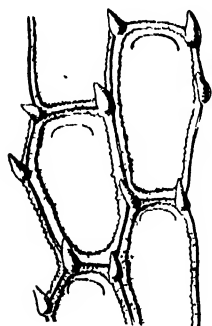
Text-fig. 1. *Conopeum serrata* (HINCKS). ×20.

Family 2. ALDERINIDAE
CANU et BASSLER 1917.
Genus 2. ELLISINA NORMAN 1903.

2. Ellisina crenulata, n. sp.
(Plate IV, fig. 1.)

Diagnosis: Zoarium incrusting, forming light greyish yellow and somewhat thick patches closely adherent to shells or stones. Zooecia alternate, arranged in lines radiating from the centre of the zoarium, somewhat large, oval or elongate. Aperture occupying the whole of the gymnocyst, closed by a membranous front wall; peristome somewhat raised, unarmed, with a calcareous, minutely crenated mural rim. Avicularia large, placed just below the lower margin of the aperture, with an elongated triangular mandible, its tip somewhat raised and directed obliquely upward. Ooecium small, transversely narrowed, quadrangular in form with a marginally thickened arched surface.

The present new species was collected at Ōma (Station 104; Sp. no. 2073), incrusting a shell of Haliotis. This is somewhat allied to *Membranipora occulata* ROBERTSON [1] but differs from it by the absence of the oral spines and scattered spatulate avicularium as well as in the ooecial features.

Family 3. BUGULIDAE GRAY 1848.
Genus 3. BUGULA OKEN 1815.

[1] ROBERTSON, 1908, Univ. Calif. Pub. Zool., Vol, 4, pp. 262-263, pl. 14, figs. 6-8.

3. Bugula neritina (LINNAEUS).
(Plate I, fig. 1.)

Sertularia neritina LINNAEUS, 1758, Syst. Nat., ed. x, 38.

Bugula neritina OKEN, 1815, Lehl. der Nat., Abt. 2. — HELLER, 1867, Ad. Bry., p. 90. — McCOY, 1881, Prod. Zool. Vict., decade vi, p. 41, pl. lix, fig. 7. — BUSK, 1884, Chall. Rept., vol. x, pt. xxx, p. 42. – – WATERS, 1887, A. M. N. H., 5, xx, p. 91, pl. iv, figs. 3, 15 — CARNUS, 1889, Prod. Faun. Medit., vol. ii, p. 6. — ORTMANN, 1890, Arch. für Naturg. Berlin, lvi, p. 24, pl. i, fig. 17. — PHILLIPS, 1899, Willey's Zool. Res., iv, p. 440. — ROBERTSON, 1905. Univ. Calif. Pub. Zool., vol. ii, p. 266, pl. ix, fig. 47, pl. xvi, fig. 97. — CALVET, 1906, Bull. Mus. Paris, p. 12. — THORNLLY, 1907, Rec. Ind. Mus., vol. i. p. 183. — OKADA, 1918, Annot. Zool. Jap., vol. ix, pt. iv, p. 484.

Acamarchis neritina LAMOUROUX, 1816, Hist. Poly. Coral, p. 58, pl. iii, fig. 2

Cellularia neritina JOHNSTON, 1847, Brit. Z., p. 340, pl. ix, figs 3, 4.

Diagnosis: Zoarium consisting of bushy tufts, dark brown or reddish brown, often tinged with purple. Branching dichotomous. Zooecia biserial, quadrangular, truncate above; aperture occupying more than two-thirds of the gymnocyst; a short denticle at the summit of the sides of the zooecium. Ooecia conspicuous, globose, attached to the inner anterior angle of the zooecium by a short peduncle. Avicularia wanting. Rootlets forming a tuft at the base of the colony.

Several small colonies which may be identified with the above species exist in the collection. They were obtained at Yunoshima (Station 1 ; Sp. no. 460). Compared with specimens from the southern part of Japan, the zoarium of this form is much more delicate and smaller, having much slenderer branches. This is widely distributed throughout the world, and in Japan was first recorded by ORTMANN from Sagami Sea, and later by me from the southern part of Japan. This specimen likewise possesses no avicularia, as is the case with all Japanese specimens, so far as have come under my observation.

Family 4. SCRUPOCELLARIIDAE LEVINSEN 1909.
Genus 4. CABEREA LAMOUROUX 1816.

4. Caberea hataii, n. sp.
(Plate I, fig. 2; Plate IV, fig. 2; Text-fig. 2.)

Diagnosis: Zoarium an irregular flabellate tuft, attached to the substratum by numerous rootlets, 30 mm. heigh. Branches somewhat

broad, dichotomously dividing at rather wide intervals. Zooecia arranged in transverse rows of four or six, somewhat elongate, nearly uniformly wide throughout the length, apertures with a spine on each side close to the anterior margin. The scutum, short and rod-like in shape, exists on the middle of either side of the peristome. Lateral avicularia present on the outer margin of the outermost lateral zooecia, with a triangular mandible, pointed at the end. Frontal avicularia small, usually existing on both sides of the lower margin of the zooecial aperture, with a semicircular mandible, with a pointed and recurved end. Vibraculum and its chamber exhibit nearly the same features as that of *Caberea lata* B. Ooecia: somewhat small, weakly inflated, nearly semicircular, with a thick arched margin. Root-fibres occur in the same manner as in *Caberea lata* B.

Text-fig. 2. *Caberea hatau* n. sp.
a. Mandible of frontal avicularium ×200.
b. Operculum ×200.

This new species is represented in the collection by several large and small colonies which were obtained at the following localities: Off Nakanosawa (Station 80; Sp. no. 459); off Ishihama (Station 43; Sp. no. 472); off Tsubakiyama (Sp. no. 1677, 1672). They were attached to worm-tubes and stones. The present species closely resembles *Caberea lata* B.[1] and *Caberea climacina* ORTMANN[2], but differs from the former by the presence of a rod-like scutum on the margin of the peristome and from the latter by the presence of lateral avicularia and of the oral spines.

5. Caberea tenella, n. sp.
(Text-fig. 3.)

Diagnosis: Zoarium small, dichotomously branched; branches slender, delicate, nearly the same breadth throughout. Zooecia biserially arranged, short, subquadrangular; apertures elliptical, occupying about half the gymnocyst of the zooecium with a narrow smooth margin,

[1] BUSK, 1852, Cat. Mar. Poly. Brit. Mus., 1, p. 39, pl. 47.
[2] ORTMANN, 1890, Arch. für Naturg. Berlin, Bd. 1, p. 22, pl. 1, fig. 6.

sloping outwards, and with one or two spines above on the outer side. Scutum large, elliptical, covering the aperture. Lateral avicularia wanting. Frontal avicularia in two forms; the larger one exists unfrequently on some zooecia with triangular, curved mandible, prominently raised, the small one more frequently appears on zooecia, placed on a lateral side of zooecial aperture, raised with a semicircular mandible, directed downwards. Vibraculum long, serrate. Ooecia unknown.

A small colony of this new species exists in the collection. It was collected at Ôma (Station 104; Sp. no. 2110), attached to a stone.

Text-fig. 3. *Caberea tenella* n. sp.
a. Ventral view of zooecia ×65.
b. Dorsal view of vibracula ×65.

Genus 5. **MENIPEA** LAMOUROUX 1816.

6. Menipea occidentalis var. catalinensis ROBERTSON.

(Plate I, fig. 3.)

Menipea occidentalis var. *catalinensis* ROBERTSON, 1905, Pub. Univ. Calif. Zool., vol. 2, no. 5, pp 255–256, pl. vii, figs 26–27. — OKADA, 1918, Annot. Zool. Jap., vol. ix, part. iv, pp. 409–410.

Diagnosis: Zoarium forming a bushy tuft, attached by a large number of root fibres. Branching regular, each tuft or frond consisting of a main rib from which secondary branches arise alternately, these again giving off tertiary branches. Internodes consisting of four or five zooecia. Zooecia elongated, narrowed below; aperture occupying about half the gymnocyst, surrounded by six jointed spines, sometimes by five or seven. Scutum fan-shaped, the edge being divided and extended into five or more spinous processes, making it so large as to cover the lower half of the aperture. Ooecia large, globose, just above the gymnocyst, perforated by a small number of pores. Root-fibres arising in root-chambers just above the lateral avicularia; the root-chambers situated on the lower zooecia only, rounded, projecting dorsally and laterally.

Several colonies which may be identified with the above species,

exist in the collection. They were obtained at near the Biological Station (Station 4; Sp. no. 425); at Ôma (Station 104; Sp. no. ?), off the Tuchiya coast (Sp. no. 1884); in front of the Biological Station (Sp. no. 1876), attached to sea-weeds.

According to ROBERTSON's description of the present form *Menipea occidentalis* var. *catalinensis* is characteristic in having a divided scutum and in the number of zooecia in an internode. But from the observation of the specimens before me, the characters above mentioned do not constantly appear, though they are variable in shape, being frequently divided, and occasionally undivided in the zooecia of an internode.

Family 4. AETEIDAE SMITT 1867.
Genus 6. AETEA LAMOUROUX 1812.

7. Aetea anguina (LINNAEUS).
(Text-fig. 4.)

Sertularia anguina LINNAEUS, 1758, Syst. Nat., Ed. x, vol. 1.
Cellaria anguina ELLIS et SOLANDER, 1786, Nat. Hist. Zooph., p. 26.
Aetea anguina LAMOUROUX, 1812, Bull. Sc. Soc. Phil., p. 184. — BUSK, 1852, Cat. Mar. Poly. Brit. Mus., p. 31, pl. xv, fig. 1. — HINCKS, 1880, Brit. Mar. Poly., p. 4, pl. 1, figs. 4, 5. — BUSK, 1884, Chall. Rep., p. 2. — OSBURN, 1912, Bull. Bur. Fish., p. 220, pl. xxi, fig. 14, 14 a. — WATERS, 1913, Proc. Zool. Soc. London, p. 463, pl. LXIV, fig. 1, 2. — HARMER, 1926, Poly. Sib. Exp., xxviii b, pp. 194–195, pl. xiii. figs. 3, 4.
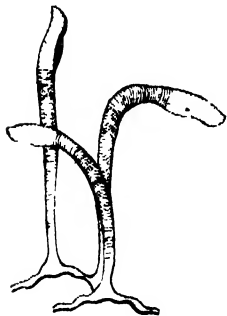
Text-fig. 4. *Aetea anguinea* (LINNAEUS) ×23.

Diagnosis: Zoarium encrusting, slender filamentous. Zooecia with a long attached portion, at first delicate and of uniform diameter, then gradually dilating and passing at a right angle into the free distal peristome, which is straight and then curved. Frontal membrane on the side facing the adherent part, expanding at its middle, the operculum terminal. Peristome at first with numerous punctations.

A large colony which may be identified with the present species exists in the collec-

tion. It was obtained at Ôma (Station 104 ; Sp. no. 2144) and attached to sea-weed. The Japanese species was previously described by HARMER from the specimen collected at a spot off Tokyo Bay collected by A. OWSTON in 1902.

Suborder **ASCOPHORA** LAMOUROUX 1909.
Family 5. **HIPPOTHOIDAE** LEVINSEN 1909.
Genus 7. HIPPOTHOA LAMOUROUX 1821.

8. Hippothoa hyalina (LINNAEUS).
(Plate I, fig 4; Plate IV, fig. 3.)

Cellepora hyalina LINNAEUS, 1766–1768, Syst. Nat. ed. 2, p. 1286.

Berenicea hyalina HASSALL, 1840, A. M. N. H., vii, p. 367.

Lepralia hyalina W. THOMPSON, 1840, A. M. N. H., v, p. 253. — JOHNSTON, 1842, Brit. Z., ed. 2, p. 301, pl. liv. fig. 1. - BUSK, 1854, Cat. Mar. Poly. Brit. Mus., ii, p 84, pl. lxxxii, figs. 1–3, pl. xcv, figs. 3–5, pl. ci. figs. 1, 2.

Mollia hyalina SMITT, 1867, Oefv. K. Vetenek. Ak. Förh., iv, 16, p. 109, pl. xxv, figs. 84, 85.

Schizoporella hyalina HINCKS, 1880, Hist. Brit. Mar. Poly., pp. 271–275, pl. xviii, figs. 8–10. — WATERS, Journ. Linn. Soc. London, v, 34, p. 20. — ROBERTSON, 1900, Proc. Wash. Acad. Sc., vol. ii, p. 326. — WHITEAVES, 1901, Geol. Surv. of Canada, p. 100. — CORNISH, 1907, Mar. Fish. Rep. Canada, no. 22, p. 77. — ROBERTSON, 1908, Univ. Calif. Pub. Zool., pp. 289–290, figs. 43–45.

Hippothoa hyalina OSBURN, 1912, Bull. Bur. Fish., xxx, pp 235–236, pl. xxiv, figs. 47, 47 a–47 c. — MARCUS, 1914–16, Bry. von den Auckland und Campbell Inseln, pp. 97–98, pl. v, fig. 3. — NORDGAARD, 1918, Bry. from the Arct. Reg., p. 52 — MARCUS, 1920, Nat. Hist. Juan Fern. & East. Isl., vol. iii, pp. 102–103, fig. 5 - MARCUS, 1921, Arkiv. für Zoologi. K. Svenska Vet., Bd. 14, no. 7, p. 12.

Cellepora hyalina NORDGARD, 1900, Den Nors. Nordh. Exp., xxvii, p. 10.

Diagnosis : Zoarium a delicate, foliaceous, irregular mass consisting of the zooecia piled on top of each other, in an irregular way. Zooecia variously shaped elongate-ovate or subcylindrical, distinct, disposed somewhat irregularly in radiating rows and separated by large punctures ; gymnocysts thin, delicate, hyaline, smooth and shining, or slightly followed transversely and sometimes with a distinct broad umbo just below the aperture. Zooecial aperture terminal, orbicular, with a deep rimule at the middle of the lower margin. Ooecia large, globose, prominent, punctured sparsely, sometimes with an umbo in the middle of the ooecial wall. Avicularia wanting.

Numerous colonies which may be referable to the above species exist in the collection. They were obtained from the following various localities: at Futagojima (Station 26; Sp. no. 430, 612); off Itanozaki (Station 31; Sp. no. 434, 445); off Ishihama (Station 43; Sp. no. 615); off Shimizugawa (Station 73; Sp. no. 627); off Yokohama (Station 81; Sp. no. 431). They were attached to individuals of sea-weed or Hydrozoa.

Family 6. ESCHARELLIDAE LEVINSEN 1909.
Subfamily a. SCHIZOPORELLAE CANU et BASSLER 1917.
Genus 9. SCHIZOMAVELLA CANU et BASSLER 1917.

9. Schizomavella auriculata (HASSALL).
(Text-fig. 5.)

Lepralia auriculata HASSALL, 1842, A. M N. H., vii, 412. — JOHNSTON, 1847, Brit. Z. ed. 2, 310, pl. liv. fig. 8. — BUSK, 1854, Cat Mar. Poly. Brit. Mus., ii, 67, pl. lxxxix, figs. 1-6.

Escharella auriculata SMITT, 1867, Oefv. K. Vetensk- Ak. Förh., iv, 12 & 90, pl. xxiv. figs. 58, 59.

Text-fig. 5. *Schizomavella auriculata* (HASSALL). ×140.

Schizoporella auriculata HINCKS, 1880, Hist. Brit. Mar. Poly., 260-263, pl. xxix, figs. 3-9. — ROBERTSON, 1908, Univ. Calif. Pub. Zool., vol. 4, 286, pl. 19, fig. 39. - OKADA, 1923, Annot. Zool. Jap., vol. 10, Art 22, p. 230.

Diagnosis: Zoarium incrusting, spreading in subcircular patches. Zooecia rhomboid, sometimes subovate, short, disposed in linear series, radiating from a central point, separated by raised lines, punctured or granular; aperture small, suborbicular, with a rimule below and with 2-4 marginal spines. A small frontal avicularium with a rounded mandible, immediately below the middle of the lower margin, usually on a mamillary eminence which is sometimes prolonged into a mucro. Ooecia subglobose, depressed, closely united to the zooecium above, sometimes completely immersed, punc-

tured, often partially enveloped in a glandular crust; occasionally mucronate.

A small colony was obtained at Ôma (Station 101; Sp. no. 2113). It was attached to a stone and is milky white in alcohol.

10. Schizomavella galeata (BUSK).
(Plate I, fig. 5; Plate IV, fig. 1, Text-fig. 6.)

Lepralia galeata BUSK, 1854, Cat. Mar. Poly. Brit. Mus., pt. II, p. 66, pl. XCIV, figs. 1. 2.

Diagnosis: Zoarium incrusting. Zooecia ovato-ventricose, disposed in linear series, separated by raised lines; gymnocyst punctured all over. Zooecial aperture orbicular, weakly contracted below by two cardelles, the lower margin slightly curved outwards, unarmed. Frontal avicularia immediately below the zooecial aperture, with semicircular mandible. Ooecia globose, inflated distinctly, covered with punctures of different sizes.

This species is represented in the collection by a few colonies and fragments. They were obtained from the following localities: Off Yunoshima (Station 6; Sp. no. 608); near Mourakojima (Station 24; Sp. no. 454); at Futagojima (Station 26; Sp. no. 623); off Yadonobe (Station 63; Sp. no. ?); off Karibazawa (Station 74; Sp. no. 613); off Akemae (Station 76; Sp. no. 611). They were attached to Hydrozoa, a plate of *Cirriped* and to a *Retepora terebrata* BUCH., and infrequently exhibit a thick lamella-like

Text-fig. 6. *Schizomavella galeata* (BUSK).
a. Mandible of frontal avicularium ×200.
b. Operculum ×200.

zoarium the base of which is attached to a substratum. The zoarium in alcohol is yellowish brown. Operculum oblong with thickened outer margin. The mandible of the avicularia shows a somewhat obtusely triangular shape, with a narrow thickened margin. The oral avicularia are not found on the marginal zooecia of a colony.

Genus 10. SCHIZOPODRELLA CANU et BASSLER 1917.

11. Schizopodrella unicornis (JOHNSTON).
(Text-fig. 7)

Lepralia unicornis JOHNSTON, 1847, Brit. Z., p. 320, pl. LVII, fig. 1. — BUSK, 1856, Quart. Journ. Mic. Sc., iv, p. 309, pl. x, fig. 3, 4. — BUSK, 1859, Crag Poly., p. 45, pl. v, fig. 4.

Schizoporella unicornis HINCKS, 1880, Hist. Brit. Mar. Poly., p. 238, pl. 35, fig. 1-5. — NORDGAARD, 1905, Hyd. Biol. Inv. Norw. Fjords, p. 165, pl. 5, figs. 23-25, 27. — CALVET, 1907, Exped. Sc. Trav. Talisman, vol. viii, p. 417. — NORMAN, 1909, Journ. Linn. Soc. London, vol. xxx, p. 417. — WATERS, 1909, Journ. Linn. Soc., Zool., vol. xxxi, p. 143, pl. xii, figs. 12, 13. — OSBURN, 1912, Bull. Bur. of Fish., 236, pl. xxv, fig. 48-48 e, pl. xxx, fig. 91. — WATERS, 1913, Proc. Zool. Soc. London, p. 501-502. — NORDGAARD, 1917, Bry. from the Arct. Reg., 56. — WATERS, 1918, Journ. Linn. Soc. London, pp. 14-15, pl. 2, fig. 14-17, 22.
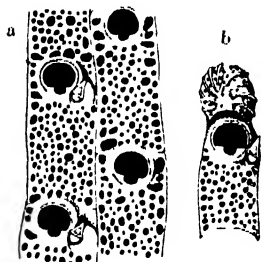
Text-fig. 7. *Schizopodrella unicornis.* (JOHNSTON).
a. Ventral view of zooecia ×20.
b. Zooecium with an ooecium ×20.

Diagnosis: Zoarium forming a greyish incrustation. Zooecia elongated squarish, bordered with a thick margin; which have no apparent order of arrangement, disposed in linear series. Zooecial aperture subcircular, arched above, slightly contracted in the middle, with two cardelles on each, the lower border with a rather large rounded rimule, peristome somewhat thick and elevated. Avicularia wanting. Ooecia subglobose, inflated above, punctured irregularly with large and small pores.

Several small and large colonies were obtained off Yunoshima Station 6; (Sp. no. 478) and off Akemae (Station 76; Sp. no. 482 a). They were attached to stones and shells of Cirripedia.

Genus 11. SCHIZOPORELLA HINCKS 1877.

12. Schizoporella crustacea (SMITT).
(Plate I, fig. 6; Plate IV, fig. 5.)

Myriozoum crustaceum SMITT, 1867, Oefv. K. Vetensk. Akad. Förh., p. 18, pl.

xxv, figs. 88–91. — RIDLEY, 1881, A. M N. H., 5, vii, p. 448. — BIDENKAP, 1898, Zool. Jahrb., vol. x, p. 622.

  Schizoporella crustacea LORENZ, 1886, Bry. von Jan. Mayen, p. 87, pl. vii, fig. 2. — WATERS, 1900, Journ. Linn. Soc., xxviii, pp. 64–65, pl. 8, figs. 11–13.

Diagnosis: Zoarium incrusting. Zooecia somewhat small, irregular in shape, very indistinctly bordered with marginal lines, disposed ·quinquncially. Zooecial aperture semicircular, with a small, somewhat deep rimule on the centre of the straight lower margin. The surface of gymnocyst perforated with small, variously sized pores in an irregular manner. Frontal avicularia frequently occur, oval, with a semicircular mandible, situated on either side of the zooecial aperture.

  Numerous colonies were attached to stones. They were obtained at Ôma (Station 104; Sp. no. 2134) and are light blue or brownish white in alcohol.

.

Subfamily b. **HIPPOPORAE** CANU et BASSLER 1917.
  Genus 12.   HIPPOPONELLA CANU et BASSLER 1920.

13. Hippoponella hippopus (SMITT).
(Plate IV, fig. 6; Text-fig. 8)

  Lepralia hippopus SMITT, 1867, Oefv. K. Vetensk. Ak. Förh., p. 20, tab. xxvi, figs. 99–105.

  Lepralia adpressa BUSK, 1852, Cat. Mar. Poly. Brit. Mus., pt. ii, p 82, tab. cii, figs. 3, 4. — BUSK, 1856, Quart. Journ. Mic. Sc., vol. iv, p. 178 — HINCKS, 1861, A. M. N. H., ser. 3, vol. ix, p. 205.

  Hippoponella hippopus BASSLER et CANU, 1920, Bull. 106, Unit Stat. Nat. Mus., p. 379.

Diagnosis: Zoarium incrusting. Zooecia somewhat oval, quincuncial; gymnocyst nodulous, with areolae along the outer margin, infrequently a weakly raised umbo, just below the zooecial aperture; zooecial aperture semicircular above, contracted on each side below the middle and margined with a weakly thickened surface. Avicularia occasionally occur on one side of the aperture with a triangular mandible. Ooecia immersed, small globose, with a longitudinal short rod-like opening in the middle of the lower margin.

  A somewhat large colony which may be referable to the above species exists in the collection. It was collected near off Itanozaki
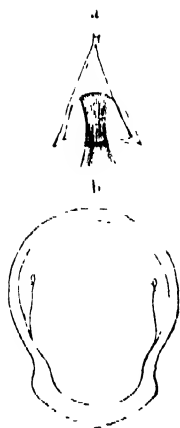
Text-fig. 8. *Hippoponella hippopus* (SMITH)
a. Mandible of frontal avicularium ×200.
b. Operculum ×200.

(Station 31 ; Sp. no. 423) and was attached to the plate of a Cirriped.

Genus 13. LEPRALIA JOHNSTON 1847.

14. Lepralia foliacea (ELLIS et SOLANDER)..
(Plate II, Fig. 1; Plate IV, fig. 7.)

Millepora foliacea ELLIS & SOL., 1786, Nat. Hist. Zooph., 133.

Eschara foliacea LAMK, 1836, An. s Vert. ed 2, II, p. 266. — MILNE-EDW, 1844, Ann. d. Sc. Nat., vi, p 36, pl. III, fig. 1 - COUCH, 1844, Corn. Faun. pt. III, p. 131 — JOHNSION, 1847, Brit Z., ed 2, p. 350, pl. LXVII — BUSK, 1852, Cat. Mar. Poly. Brit. Mus., ii p 89, pl CVI, figs. 4–7. — HELLER, 1867, Bryoz. d. adriat. Meer, p. 38 — MANZONI, 1870, Bryoz. foss Ital, contr. IV, p. 18, pl. I, fig. 4 & pl. IV, fig. 24.

Lepralia foliacea HINCKS, 1880, Hist. Brit. Mar. Poly, pp 300 302, pl. xlvii, figs. 1–4.

Diagnosis : Zoarium foliaceous, membrano-calcareous. Zooecia disposed in two layers placed back to back, or occasionally in one layer, ovate-elongate, or rhomboid, quincuncially arranged, separated by distinct lines, very moderately convex ; surface punctured and often nodulous, the punctures frequently surrounded by thick reticulated ridges sometimes areolated round the margin ; aperture arched above, weakly contracted a little below the middle, where there is a small cardelle on each side, the lower margin almost straight, or very slightly elevated in the centre. A prominent central avicularium just immediately below the lower apertural margin has a rounded mandible pointing downwards, sometimes replaced by a spatulate avicularium. Ooecia large, often subimmersed, a little flattened in front, smooth and shining.

Several small colonies which may be identified with the above species exist in the collection. They were attached to seaweed and were obtained off Yunoshima (Station 6 ; Sp. no. 645) and off Futagojima (Station 26 ; Sp. no. unknown).

15. Lepralia pertusa (ESPER).

(Plate II, fig. 2; Plate IV, fig. 8; Text-fig. 9.)

Cellepora pertusa ESPER, 1791, Pflanz. Cellep., p. 119, pl. x, fig. 2

Escharina pertusa MILNE-EDWARDS, 1844, Lamk An s. Vert., ed. 2, ii. p 232

Cellepora perlacea W. THOMPSON, 1843, A. M. N H., x p. 20

Lepralia pertusa JOHNSTON, 1847, Brit. Z, ed. 2, p. 311, pl. liv. fig. 10. - BUSK, 1852, Cat. Mar. Poly. Brit. Mus, ii, p 80, pl lxxviii, fig 3, pl. lxxix, figs 1, 2. – HELLER, 1867, Bryoz. d Adr. Meeres, p. 35.

Escharella pertusa SMITT, 1872, Flor. Bryoz., pl ii, p. 55

Diagnosis: Zoarium incrusting. Zooecia large, regularly ovate, distinct, separated by raised lines; gymnocyst covered with punctures; zooecial aperture suborbicular, contracted below with two lateral cardelles, the lower margin slightly curved outwards; usually a tubercle below the aperture, which sometimes rises into a well developed mucro; the peristome slightly thickened, unarmed. Frequently frontal avicularia on one side, a little below the aperture, with triangular mandible directed obliquely upwards. Ooecia globose, somewhat depressed in front, thickly covered with punctures of different sizes.

There exist in the collection numerous colonies which may be identified with the above species. They were obtained from the following localities: Yunoshima (Station 1; Sp. no. 483, 638); off Yunoshima (Station 6; Sp. no. 600, 606, 618); off Hanaguri (Station 21; Sp. no. 611), off Itanozaki (Station 31; Sp. no. 423); off Namiuchi (Station 50; Sp. no. 455). They are attached to a stone, a plate of Cirriped and bivalve shells.

Text-fig. 9 *Lepralia pertusa* (ESPER).
a Mandible of frontal avicularium ×200
b Operculum ×200.

In the specimens on hand, two frontal avicularia are usually found on both sides of the zooecial aperture, but infrequently there is only one, or rarely none. The specimen from station 50 (Sp. no. 455), is provided with a somewhat prominent median projection on the frontal surface, just below the lower margin of the zooecial aperture.

16. Lepralia reticulata, n. sp.
(Plate V, fig. 4, 5.)

Diagnosis: Zoaria encrusting, forming an irregular crest. Zooecia large, quadrangular or elongated quadrangular, punctured over the entire gymnocyst with rather large pores, with somewhat elevated thick margin, disposed in lines. Zooecial aperture somewhat large, broader than long, arched above, weakly contracted on each side below the middle, lower margin nearly straight, peristome slightly thickened and elevated, forming a narrow thick border round the aperture. A small bluntly pointed umbo presents below the lower margin of the zooecial aperture. Marginal zooecia without the aperture, entirely perforated with large pores on the surface. Avicularia and ooecia wanting.

Small and large colonies which may be referable to the above species exist in the collection. They were obtained off Namiuchi (Station 50; Sp. no. 455); at Ôma (Station 104; Sp. no. 2107, 2143) and were attached to coral and shells.

It is worthy to note that the marginal zooecia of the large zoarium show a different zooecial feature from that of the ordinal inner ones. Those are not provided with zooecial apertures (as in the kenozooecia of *Adeonella*), being entirely perforated with large pores, formed a complex network on the frontal surface.

17. Lepralia bilabiata HINCKS.
(Plate II, fig. 3; Text-fig. 10.)

Lepralia bilabiata HINCKS, 1881, A. M. N. H., vol. 13, p. 49, pl 3, figs. 1, 1a, 1b. — ROBERTSON, 1908, Univ. Calif. Pub., Zool. vol. 4, no. 5, pp. 298–300, pl. 21, figs. 61-64.

Diagnosis: Zoarium incrusting. Zooecia short and broad, alternate, rounded above, truncate below; gymnocyst at the outer growing rim almost wholly membranous soon covered over somewhat more than half its surface by a delicate calcareous front wall, convex, slightly granular; in older zooecia, rising into a keel, extending from the lower rim of the operculum to the base of the zooecium, and forming a thick rim, above the large operculum; frontal wall marked by radiating striae and sometimes rising into a distinct umbo. Operculum
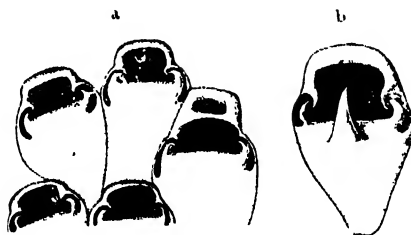
Text-fig. 10. *Lepralia bilabiata* HINCKS.
a. Few zooecia, one of them bears an ooecium ×20
b. Zooecium with a spine on frontal wall × 40.

large, brown, occupying almost half the gymnocyst of the zooecium, semicircular above, curved on the lower margin. Ooecia immersed, formed by an extension of the zooecial wall above the operculum and marked by a large pore, covered with a chitinous membrane. No avicularia.

Numerous colonies in the collection are referable to the above species. They were obtained from the following localities: Yunoshima (Station 1; Sp. no. 480); off Yunoshima (Station 6; Sp. no. 619); off Hanaguri (Station 21; Sp. no. 452), off Itanozaki (Station 30; Sp. no. 462); off Itanozaki (Station 31; Sp. no. 423, 439); off Yadonobe (Station 63; Sp. no. 657, 428. 2255); Asadokoro (Station 72; Sp. no. 465); off Fukkoshi (Station 79; Sp. no. 473). They were attached to dead bivalve shells and shell plates of Cirripeds. Most of the specimens are provided with prominent spinous projections, conically pointed at the end, on the middle of the gymnocyst, just below the lower margin of the zooecial aperture.

Genus 14. HIPPODIOPLOSIA CANU 1916.

18. Hippodiplosia pallasiana (MOLL).
(Plate II, fig. 4; Plate V, fig. 1.)

Eschara pallasiana MOLL, 1803, Seerinde, p. 64, pl 10, fig. 13.
Cellepora pallasiana LAMOUROUX, 1816, Hist. Pol. corall flex., p. 95, no. 190.
Lepralia pallasiana BUSK, 1854, Cat. Mar. Pol. Brit. Mus., II, p. 81, pl. lxxxiii. figs. 1, 2. — BUSK, 1859, Crag Pol., p. 54, pl. IX, fig. 7. — BUSK, 1863, Quart. Journ. Mic. Sc., iv, p. 309, pl. xi. figs. 1, 2. — HINCKS, 1861, A. M. N. H, 3, IX, p. 204. — SMITT, 1868, Krit Fört. Skand. Haf. Bry., IV, pp. 19, 123, pl. xxvi, fig. 93. — HINCKS,

1890, Hist. Brit. Mar. Poly., pp, 297–299, pl. xxxiii. figs. 1–3; pl. xxiv, fig. 4. — CORNISH, 1907, Mar. Fish Rep. Canada, no. 22, p. 77. – OSBURN, 1912, Bull. Bur. Fish., vol. xxx, p. 210, pl. xxv, fig. 51, pl. xxx, fig. 89.

Diagnosis : Zoarium incrusting. Zooecia large, broad-ovate, distinct, coarsely punctured along the entire outer margin of the gymnocyst, disposed in lines ; zooecial aperture ample, longer than broad, arched above, contracted on each side below the middle, lower margin slightly curved outwards ; the peristome unarmed, thickened ; commonly an umbo below the lower margin of the zooecial aperture. Ooecia unknown.

Three small and large colonies in the collection are referable to the above species. They were obtained at the following localities : off Yunoshima (Station 6 ; Sp. no. 606) ; off Yadonobe (Station 63 ; Sp. no. 436) ; off Tozawa (Station 66 ; Sp. no. 440). They were attached to stones and to plates of Cirripeds.

In most specimens, the zooecia are provided with a median process on the gymnocyst, somewhat below the lower margin of the zooecial aperture, but in most of these unfrequently it is entirely wanting.

Subfamily c.   **MICROPORELLAE** CANU et BASSLER 1917.
Genus 15.   **MICROPORELLA** HINCKS 1877.

19.   Microporella ciliata PALLAS.
(Plate H, fig. 5, text-fig. 11.)

Eschara ciliata var B, PALLAS, 1766, ELENCH. Zooph., p. 38.

Cellepora cilliata LINN , 1766, Syst. Nat., ed. 12, p. 1286.

Lepralia cilliata JOHNSTON, 1847, Brit. Z., ed. 2, p 323, pl. lvii. figs. 4, 5. — BUSK, 1854, Cat. Mar. Poly. Brit. Mus., ii, p 73, pl. lxxii, figs. 1, 2, pl. lxxvii, figs. 3, 4, 5. - · BUSK, 1859, Crag Pol , p. 42, pl. vii. fig. 6

Porina ciliata SMITT, 1867, Oefv. K. Vetensk. Ak. Förh., pt. ii. p. 26, pl. vi. figs. 126–129.

Microporella ciliata HINCKS, 1880, Hist Brit. Mar. Poly., pp. 206–210, pl. xxviii, figs. 1 8. — NORDGARD, 1918, Bry. from the Arct Reg., pp. 59- 60. — OKADA, 1923, Annot. Zool. Jap., vol. 10, Art. 22, p 227.

Eschara ciliata PALLAS, 1766, Elenchus Zoophy., p. 38.

Microporella vibraculifera HINCKS, 1883, A. M. N. H., 5, xi, p. 443, pl. xvii, fig. 2.

Diagnosis : Zooecia ovate, obscurely hexagonal, convex, separated

by well-marked sutures and somewhat distinctly punctate, or granular on the gymnocyst, quincuncial, or disposed in radiating lines ; zooecial aperture semicircular above, with a slight rim, the lower margin straight, the upper with from five to seven long spines ; median pore lunate, a little below the zooecial aperture, frequently borns on a prominent mucro, or sometimes almost hidden behind it. Frontal avicularia on one side or the other, with an acute mandible, directed obliquely upwards or lateral-wards, which is often prolonged into a slender vibraculoid shape. Ooecia globose, areolated round the base, minutely punctate or granular ; two spines visible in front of it.
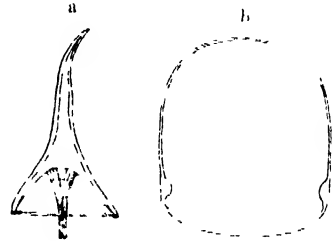
Text-fig 11. *Microporella ciliata* PALLAS.
a. Mandible of frontal avicularium ×200
b. Operculum ×200.

A few small colonies in the collection are referable to the above species. They were collected at the following localities : Off Yunoshima (Station 6 ; Sp. no. 609) ; Ôshima (Station 15 ; Sp. no. 479) ; off Mourakojima (Station 22 ; Sp. No. 449) ; off Itanozaki (Station 31 ; Sp. no. 642) ; off Jukunohe (Station 63 ; Sp. no. 603) ; Ôma (Station 101 ; Sp. no. 609, 2090) together with *Microporella malusii* (AUD). They were attached to a stem Hydrozoa, stones, a species of sea-weed, Zostera, and to Arca.

20. Microporella maiusii (AUDOUIN).
(Text-fig. 12.)

Cellepora malusii AUDOUIN & SAVIGNY, 1811, p. 239, pl. 8, fig 8.

Lepralia malusii JOHNSTON, 1847, Brit Z , ed 2, p 314, pl. 55, fig. 4. - - BUSK, 1854, Cat. Mar. Poly. Brit. Mus., pt 2, p 83, pl. 103, figs 1-4.— BUSK, 1859, Crag Pol., p. 53, pl. 8, fig. 3. — WATERS, 1879, A. M. N. H., vol. 3, p. 33.

Reptoporina malusii D'ORBIGNY, 1850-52, Pal. Fran. Terr. Cret , vol. 5, p 443.

Microporella malusii HINCKS, 1880, Hist Brit Mar Poly., p. 211, pl. 28, figs. 9–11, pl. 29, figs. 1, 2. — BUSK, 1884, Chall Rep., pl. 30, vol. 10, p 137 — ROBERTSON, 1908, Univ. Pub. Calif. Zool., vol. 4, no. 5, pp. 282-283, pl. 18 figs. 35–36.

Fenestrulina malusii JULLIEN, 1888, Miss. Scient. Cap Horn , T 6, p. 38, pl. 15, figs. 1-3.

Diagnosis: Zoarium incrusting. Zooecia ovate or rhombic, alternate, radiating from a central part; gymnocyst calcareous, punctate, slightly convex; immediately inside the margin a row of stellate pores;
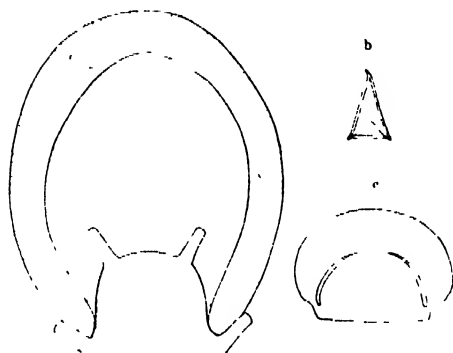
Text-fig. 12. *Microporella malusii* (AUDOUIN).
a. Ooecium seen from the surface × 150.
b. Mandible of avicularium × 150.
c. Operculum × 150.

the center of the gymnocyst occupied by a large lunate, toothed pore. Zooecial aperture straight below, semi-circular above, with four or five marginal spines. Ooecia globose, surrounded by an areolated border.

Of this species, there exist in the collection numerous colonies attached to seaweeds. They were obtained off Hanaguri (Station 21 ; Sp. no. 452).

Family 7. SMITTINIDAE LEVINSEN 1909.
Genus 16. SMITTINA NORMAN 1909.

21. Smittina trispinosa (JOHNSTON).
(Text-fig. 13.)

Discopora trispinosa JOHNSTON, 1838, Ed. Phil. Journ., xiii, p. 322.
Lepralia trispinosa JOHNSTON, 1849, Brit. Z., ed. 2, p. 324, pl. lvii, fig. 7, — BUSK, 1854, Cat. Mar. Poly. Brit. Mus., ii, p. 70, pl. lxxxv, figs. 1, 2, pl. xcviii, pl. cii, fig. 2. — HINCKS, 1877, A. M. N. H., p. 100, pl. xi, fig. 1. — DARWSON, 1859, Geol. Surv.

Canada, p. 256. — PACKWARD, 1867, Proc. Bost. Soc. Nat. Hist., vol. 1, p 67. — WHITEAVES, 1901, Geol. Surv. Canada, p. 106.

Smittia trispinosa HINCKS, 1880, Hist. Brit. Mar. Poly., pp. 353-356, pl. xLix, figs. 1-8. — OSBURN, 1912, Bull. Bur. Fish., xxx, p. 246, pl. xxvii, figs. 65, 65 a. — NORDGAARD, 1900, Den. Nors. Nordh. Exp., xxvii, p. 13, pl. 1, fig. 9. — NORDGAARD, 1918, Bry. from the Arct. Reg., pp. 61-62. — OKADA, 1923, Annot. Zool. Jap., vol. 10, art. 22, p. 228.

Diagnosis: Zoarium encrusting on stones, shells, etc. Zooecia somewhat ovate or elongate ovate, sometimes nearly rectangular, disposed in linear series or quincuncially, separated by raised lines and punctured round the gymnocyst. Zooecial aperture suborbicular; peristome somewhat raised, with
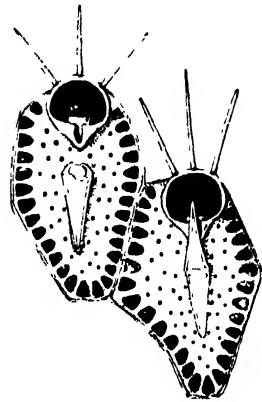
Text-fig. 13. *Smittina trispinosa* (JOHNSTON). ×40.

a broad deep rimule, a broad lyrula within the lower margin; oral spines 2-4. Avicularia somewhat large, situated a little below the zooecial aperture with an acute mandible directed upwards. Ooecia large, ample, globose, somewhat flattened in front, usually with two or three large pyriform punctures.

A somewhat large colony which may be identified with the above species exists in the collection. It was obtained at Ôma (Station 104; Sp. no. 2143), attached to a stone.

22. **Smittina reticulata** (MACGILLIVRAY).
(Text-fig 14.)

Lepralia reticulata MACGILL., 1842, A. M. N. H., ix, p. 467. — JOHNSTON, 1847, Brit. Z., ed. 2, p. 317, pl. Lv, fig. 10. — BUSK, 1854, Cat. Mar. Poly. Brit. Mus., ii, p. 66, pl. xc, fig. 1, pl. xcii, figs. 1, 2.

Smittia reticulata HINCKS, 1880, Hist. Brit. Mar. Poly., pp. 346-348, pl. xlviii, figs. 1-5. — NORDGARD, 1918, Bry. from the Arct. Reg. p. 60. — NORDGARD, 1900, Den. Nors. Nordh.-Exp., xxvii, p. 13.

Diagnosis: Zoarium incrusting. Zooecia ovate-elongate, in linear series, separated by raised lines, strongly areolated round the margin of the gymnocyst, smooth or slightly roughened. Zooecial aperture
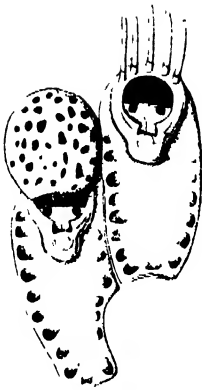
Text fig. 14. *Smittina 're-ticulata* MacGill. Two zooecia one of them with an ooecium ×65.

orbicular, with a thin raised cryptocyst and a deeply channelled rimule in the lower lip, lyrula rod-like, largest, and cardelles small. Avicularia with an acute mandible pointed downwards, situated immediately below the sinus. Ooecia semicircular or globose, prominent, punctured, the elevated peristome in front giving them a hooded appearance.

A complete circular colony which may be identified with the above species exists in the collection. It was obtained off Yuno-shima, attached to Ascidea (*Chelyosoma siboya* OKA).

Genus 17. MUCRONELLA HINCKS 1880.

23. Mucronella hozawai, n. sp.
(Plate II, fig. 6; Plate V, fig. 2; Text-fig. 15.)

Diagnosis: Zoarium encrusting on seaweeds, forming a thin, nearly circular crest. Zooecia elongated oval, distinctly separated,

Text-fig. 15. *Mucronella hozawai* n. sp.
a. Few zooecia one of them with an ooecium ×20.
b. Side view of zooecium × 10.

disposed in radiating rows or in linear series, punctured sparsely on the gymnocyst except along the zooecial aperture. Zooecial aperture somewhat semi-circular, arched above, and the lower margin nearly straight, with a distinct broad elevated cryptocyst. Avicularia wanting. Ooecia prominent, globular, unpunctured, with a small umbo on the centre and with the indistinct radiating lines on the outer margin. Two curved spines are projected backwards from both corners of

the lower margin, the ends often crossing in the middle.

Several colonies of this new species exist in the collection. They were obtained at Kawauchi (Station 64; Sp. no. 641), off Tozawa (Station 66; Sp. no. 635) and were attached to *Zostera* and other sea-weeds.

24. Mucronella takatukii, n. sp.
(Text-fig. 16.)

Diagnosis: Zoaria incrusting on sea-weeds or substratum. Zooecia tubular, arranged in linear series, gymnocyst thin, delicate; zooecial aperture circular with an elevated lower margin, and with two long spines on the upper margin. Oral avicularium small, with pointed and curved mandible, is situated at the centre of the lower elevated margin. Ooecia unknown.

Text-fig. 16. *Mucronella takatsukii* n. sp. × 40.

Two colonies were obtained off Tozawa (Station 66; Sp. no. 622) and at Ôma (Station 101 Sp. no. 2171).

Family 8. RETEPORIDAE SMITT 1867.
Genus 18. RETEPORA IMPERATO 1599.

25. Retepora terebrata BUCHNER.
(Plate III, fig. 1; Plate V, fig. 3.)

Retepora terebrata BUCHNER, 1924, Zool. Jahrb. Syst, bd. 48, pp. 191–193, taf. 17, figs. 1–2, text-fig. n. p.

Diagnosis: Zoarium erect, forming a somewhat large convoluted mass, growing from an incrusting disk or with a short peduncle. Fenestrae oval or elliptical, about three to four times as long as wide. Zooecia somewhat large, arranged biserially in alternate arrangement; gymnocyst nearly smooth on the surface, sloping upward from the side to the median line, perforated by two or three, small, circular pores. Zooecial aperture nearly semicircular on the upper margin, with a somewhat deep rimule on the lower. Frontal avicularium

large, present on most zooecia, much elevated on centre of the gymnocyst, with a triangular, obtuse mandible directed obliquely upward. Ooecia relatively small, subspherical, smooth-surfaced, with a small peak in the middle of lower margin. Dorsal surface nodulous, frequently perforated by a few small 'pores, with somewhat irregularly developed vibices, on some of which there is an occurrence of a smaller avicularium than that of the ventral surface.

Numerous large and small colonies and fragments of the above species were obtained from following localities: off Hanaguri (Station 12; Sp. no. 435); Futagojima (Station 26; Sp. no. 647; 656); off the Biological Station (Station 28; Sp. no. 453); off Itanozaki (Station 30; Sp. no. 447, 462 (a), 471 1935); off Itanozaki (Station 31; Sp. no. 441, 465), off Ōshima (Station 39; Sp. no. 458); off Hanaguri (Station 469; Sp. no. 469); off Namiuchi (Station 51; Sp. no. 477), off Ozawa (Station 62; Sp. no. 432); off Jukunohe (Station 63; Sp. no. 428, 463), Asadokoro (Station 72; Sp. no. 467); off Shimizugawa (Sfation 73; Sp. no. 474, 4121); off Karibazawa (Station 74; Sp. no. 446, 443, 433); off Noheji (Station 75; Sp. no. 427); off Akemae (Station 76; Sp. no. 630, 637), off Fukkoshi (Station 79; Sp. no. 604, 473 a); off Ushinosawa (Station 80; Sp. no. 633); Ōma (Station 104; Sp. no. 2121); off Yunoshima (Sp. no. 2258), off Tsubakiyama (Sp. no. 1678), off Noheji (Sp. no. 1827).

They were attached to a stone, worm-tube, Hydrozoa and bivalve shell etc. It is interesting to note that the Reteporan species of Mutsu Bay is represented only by the present species.

Family 9. CELLEPORIDAE Busk 1852.
Genus 19. CELLEPORA Linnaeus 1767.

26. Cellepora incrassata Lamarck.
(Plate III, fig. 2; Plate V, fig. 6; Text-fig. 17.)

Cellepora incrassata Lamarck, 1856, Ency. méth., ed. 2, vol. 2, p. 256. — Smitt, 1867, Oefv. K. Vetensk.-Ak. Förh., p. 33, figs. 212–216. — Smitt, 1868, Kritisk För. öfver Skand. Hafs-Bry., pp. 198–200, taf. xxviii, figs. 214–215. — Hincks, 1877, A. M. N. H., vol. 19, p. 105. — Robertson, 1900, Proc. Wash. Acad. Calif. Pub., vol. 4, pp. 312–313, pl. 24, fig. 187, 88.

Diagnosis: Zoarium forming coarse, rounded nodules, incrusting

seaweed. Zooecia large, round, decumbent in young colonies becoming erect in older portions of the colony and piled one on top of the other; gymnocyst soon becoming heavily calcified and punctured with large pores. Primary orifice, rounded on the upper peristome with a well pronounced rimule on the lower peristome; secondary orifice orbicular; on each side an avicularium somewhat elevated, the two often inclined toward each other; mandible directed slightly away from the zooecial aperture; avicularia frequently lacking exception zooecia that bear ooecia. Ooecia, relatively small, decumbent,

Text-fig. 17. *Cellepora incrassata* SMITT.
a. Operculum ×200.
b. Mandible of oral avicularium ×200.
c. Mandible of frontal avicularium ×400.

rounded, smooth, broader than high, a triangular portion, in front of which it is thin, and perforated by numerous pores in radiating rows.

Numerous colonies of the above species from the following localities: Yunoshima (Station 1; Sp. no. 480), off Yunoshima (Station 6; Sp. no. 620); Ôshima (Station 15; Sp. no. 456 1597); Futagojima (Station Sp. no. ?); off Itanozaki (Station 30; Sp. no. 1935 b), off Itanozaki (Station 31; Sp. no. 423); off Ôshima (Station 39; Sp. no. 625); off Ishihama (Station 43; Sp. no. 468), off Namiuchi (Station 51; Sp. no. 614), off Akemae (Station 76; Sp. no. 482 (b), 651); off Fukkoshi (Station 79; Sp. no. 473 b); off Yokohama (Station 81; Sp. no. 431); Ôma (Station 104; Sp. no. 2169).

They form small cubical or irregular shaped masses and are attached to *Hydrozoa*, worm-tubes, stones, sea-weeds, dead bivalve shells and the thorasic appendage of crabs.

Genus 20. COSTAZZIA. NEVIANI 1895.

27. Costazzia costazi AUDOUIN.
(Plate III. figs. 3-5.)

Cellepora costazii AUDOUIN, 1826, Explic. des planch. de M. Savigny.
Cellepora costazii HINCKS, 1880, Hist. Brit. Mar. Poly., p. 411, pl. 55, fig. 11-14.
— ROBERTSON, 1908, Univ. Calif. Pub. Zool., vol. iv. pp. 313-314, pl. 21, fig. 89.

Diagnosis : Zoarium incrusting or growing in ball-like or discoidal masses. Zooecia decumbent, somewhat erect, disposed irregularly and crowded together one on top of another, gymnocyst perforated by large pores ; zooecial aperture suborbicular, with a rimule on the lower margin ; becoming deeply sunk secondarily by the growth of the peristome, bearing an erect process on each side of the aperture with a small avicularium on its summit. Interzooecial avicularia scattered irregularly among the zooecia, with a spatulate, large mandible. Ooecia decumbent, rounded, smooth, much broader than high, with an area in front pierced by large pores.

There exist numerous colonies which may be identified with the above species, in the collection. They were obtained from the following localities : Yunoshima (Station 2 ; Sp. no. 448) ; off Yunoshima (Station 6 ; Sp. no. unknown), off Hanaguri (Station 21 ; Sp. no. 452), off Futagojima (Station 26 ; Sp. no. 621, 654), off Futatsuya (Station 44 ; Sp. no. 616) ; off Wakinosawa (Station 61 ; Sp. no. 429) ; off Kozawa (Station 62 ; Sp. no. 437) ; off Tozawa (Station 66 ; Sp. no. 622) ; off Shimizugawa (Station 73 ; Sp. no. 605 unknown), off Noheji (Station 75 ; Sp. no. 424) ; off Tomarikawa (Station 78 ; Sp. no. 470) ; off Ushinosawa (Station 80 ; Sp. no. 422) ; Takaisozaki (Station 102 ; Sp. no. 2029), Ôma (Station 104 ; Sp. no. 2146), Ôma Bay (station 105 ; Sp. no. 2186).

They were attached to stones and sea-weed. It is interesting to note, that the zoarium of this collection exhibits a variable outer configuration, forming frequently a cubical mass, infrequently a discoidal convex thickened mass, and occasionally an irregular incrusting mass. Seen as to the outer configuration, they may be separated into distinct species, but from the zooecial features they are included under the same species.

September 29, 1928.

PLATE I

Fig. 1. *Bugula neritina* LINNAEUS ×1.
Fig. 2. *Caberea hatai*, n. sp. ×1.
Fig. 3. *Menipea occidentalis* var. *catalinensis* ROBERTSON ×1.
Fig. 4. *Hippothoa hyalina* (LINNAEUS) ×1.
Fig. 5. *Schizomavella galeata* (BUSK) ×1.
Fig. 6. *Schizoporella crustacea* LORENZ ×1.

PLATE II

Fig. 1. *Lepralia foliacea* (ELLIS & SOLANDER) ×2.
Fig. 2. *Lepralia pertusa* (ESPER) ×1.
Fig. 3. *Lepralia bilabiata* HINCKS ×1.
Fig. 4. *Hippodiplosia pallasiana* (MOLL) ×1.
Fig. 5. *Microporella ciliata* PALLAS ×1.
Fig 6. *Mucronella hozawai*, n. sp. ×1.

PLATE III

Fig. 1. *Retepora terebrata* BUCHNER ×1.
Fig. 2. *Cellepora incrassata* SMITT ×1.
Fig. 3. *Costazzia costazi* (AUDOUIN) ×1.
Fig. 4. *Costazzia costazi* (AUDOUIN) ×1.
Fig. 5. *Costazzia costazi* (AUDOUIN) ×1.

PLATE IV

Fig 1. *Ellisina crenulata*, n. sp. ×5.
Fig. 2. *Caberea hatai*, n. sp. ×5.
Fig. 3. *Hippothoa hyalina* (LINNAEUS) ×5.
Fig. 4. *Schizomavella galeata* (BUSK) ×5.
Fig. 5. *Schizoporella crustacea* LORENZ ×5.
Fig. 6. *Hippoponella hippopus* (SMITT) ×5.
Fig. 7. *Lepralia foliacea* (ELLIS & SOLANDER) ×5.
Fig. 8. *Lepralia pertusa* (ESPER) ×5.
Fig. 9. *Lepralia pertusa* (ESPER) ×5.

PLATE V

Fig. 1. *Hippodiplosia pallasiana* (MOLL)
Fig. 2. *Mucronella hozawai*, n. sp. ×5.
Fig. 3. *Retepora terebrata* BUCHNER ×5.
Fig. 4. *Lepralia reticulata*, n. sp. ×5.
Fig. 5. *Lepralia reticulata*, n. sp. ×5.
Fig. 6. *Cellepora incrassata* SMITT ×5.

Y. OKADA : Cheilostomatous Bryozoa of Mutsu Bay.
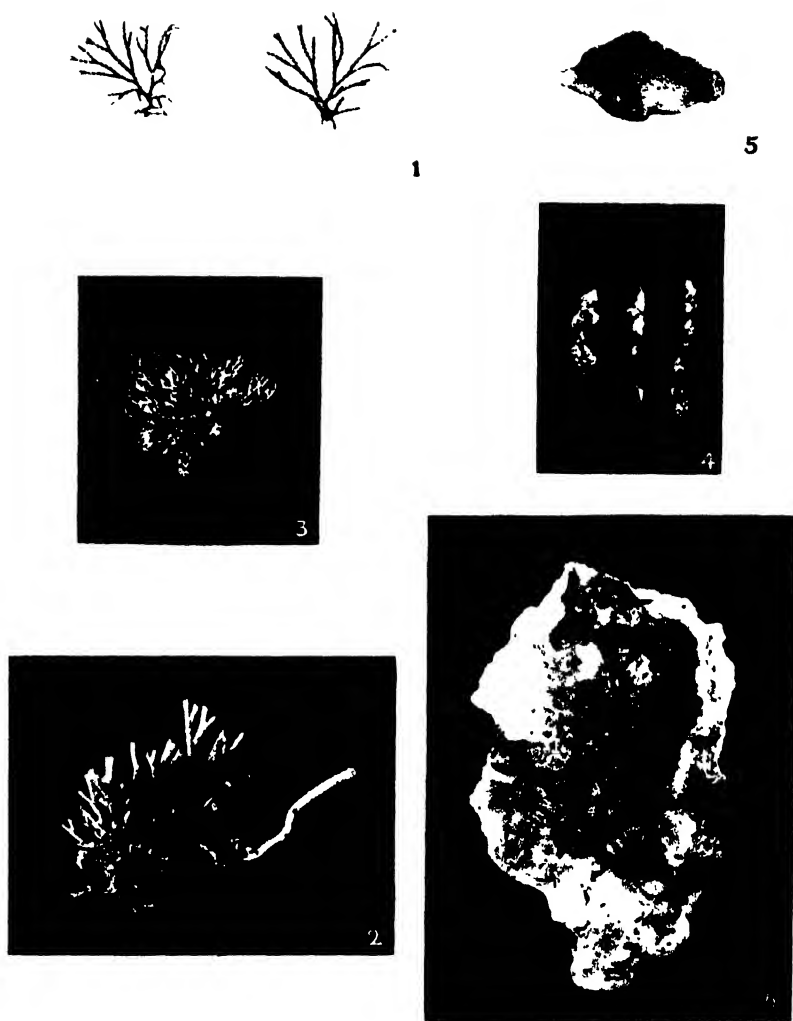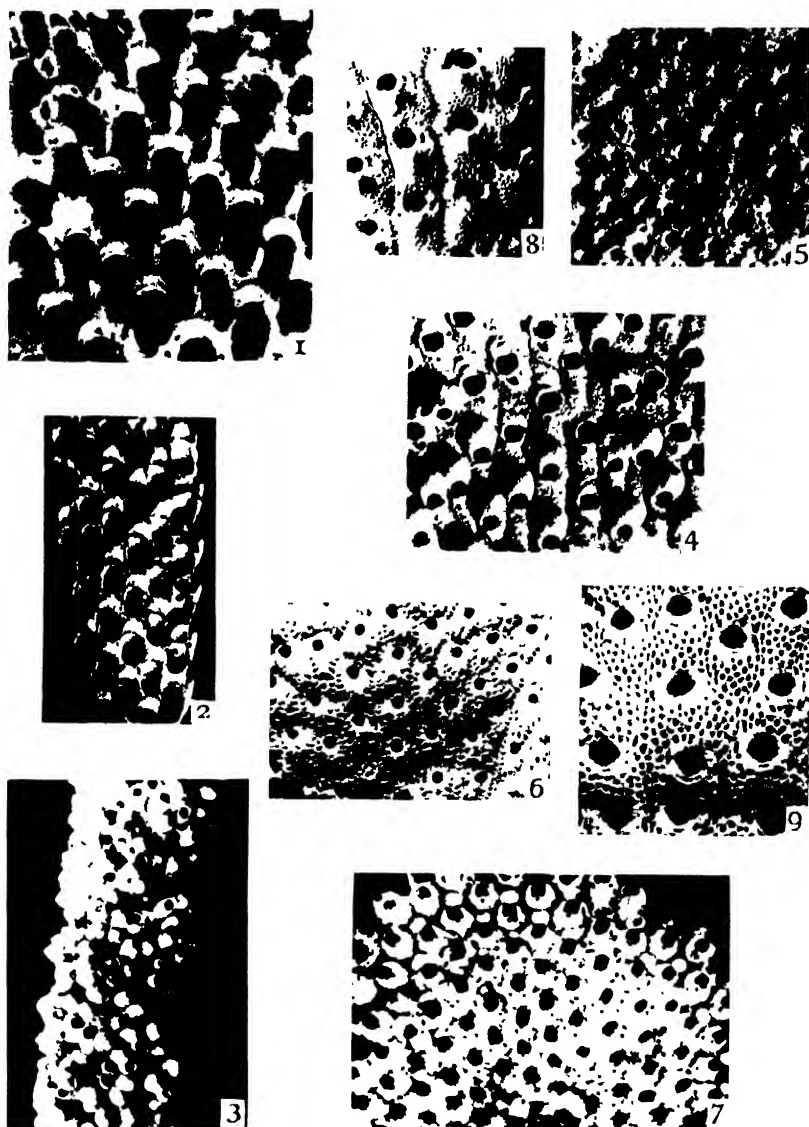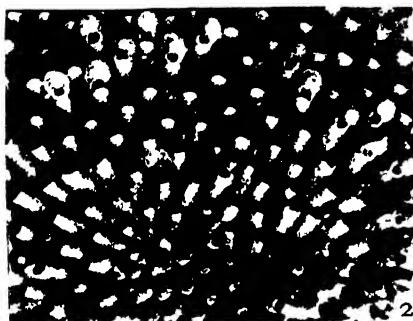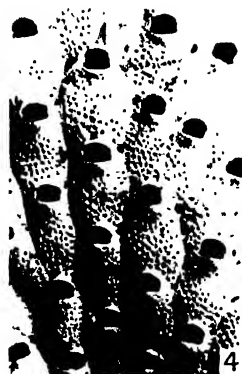
Studies on the Hepaticae of Japan. I.

By

YOSHIWO HORIKAWA.

(With Plates VI–IX and 6 Text-Figures.)

INTRODUCTION

The Hepaticae of Japan are indeed of unusual interest, as already pointed out by some authors[*]. According to our present knowledge, they include a considerably large number of species for the limited area of the Islands, over 500 species and 90 genera having already been reported in literature. Moreover, among them are included both northern and southern elements. More striking even than this is the fact that the vast majority of them are quite endemic (about 65% of the total species). Some of the most remarkable, for example, are as follows:

Funicularia japonica STEPHANI.
Plagiochasma japonica STEPHANI.
Marchantia cuneiloba STEPHANI.
Pallavicinia longispina STEPHANI.
Makinoa crispata (STEPH.) MIYAKE.
Cavicularia densa STEPHANI.
Calobryum mnioides (LINDB.) STEPHANI.
Schiffneria viridis STEPHANI.
Bazzania Pompeana (SANDE-LAC.) MITTEN.
Ptilidium Bisseti (MITT.) EVANS.
Ptilidium sacculatum (MITT.) STEPHANI.
Dendroceros japonica STEPHANI.
etc.

These most characteristic features of Japanese Hepatic-Flora are most likely due to the extended latitude of the Islands, and consequently

---

[*] MITTEN, W., Jour. Linn. Soc. Lond. Bot, Vol. VIII, p. 148 (1865) SCHIFFNER, V., Oesterr. Bot. Zeitsch., Bd. 49, p. 385 (1899); EVANS, A. W., Proc. Wash. Acad. Sci., Vol. VIII, p 141 (1906).

to the varied edaphic and atmospheric conditions which are to be found there.

Unfortunately, however, in Japan the Hepaticae have been so much neglected by collectors that our knowledge of their species and geographical distribution is still very incomplete. Up to the present, in this country, the localities in which the Hepaticae have been collected were limited to a few districts, such as Tokyo (by C. TSUGE, K. MIYAKE and T. MAKINO), the northern districts (by Abbé U. FAURIE), province Tosa (by T. MAKINO, S. OKAMURA and T. YOSHINAGA, formerly INOUE), province Rikuzen (by E. IISHIBA) and province Iyo (by K. OKUDAIRA). But even these districts have not yet been completely explored. The following are instances of this situation. *Lunularia cruciata* and *Notothylas* sp. are rather common in Tokyo, Sendai, and Hiroshima, and *Pleurozia arcuata* occurs in Formosa ; and yet none of these noteworthy genera just mentioned have ever been reported from Japan till the present time. This circumstance shows how little we know about the Hepaticae of Japan, and indicates that much still remains to be learned. The total number of species will surely become greater and greater, as the flora is more thoroughly explored in the future.

We owe our present knowledge of the Hepaticae of Japan mostly to STEPHANI. But it is to be regretted that his descriptions are based only on dried specimens sent to him from time to time from Japan for determination. Accordingly most of his descriptions are not complete and none of these species are illustrated with figures, and there are also many species described without any definite locality, date, or collector's name.

As it is clear how much remains to be done to complete our knowledge concerning the Japanese Hepaticae, it is my desire to describe, figure, and discuss in the successive series of these reports as many species as possible which occur in the Japanese Empire (Kulile Is., Sakhalin, Hokkaido, Honshiu, Shikoku, Kiushiu, Riukiu Is., Formosa, Bonin Is., Korea and etc.).

It is to be further noted that the late C. TSUGE made the first step in the study of Japanese Hepaticae, during his academic year, 1886–1887. Immediately after completing the university course, he became Professor of the Ist Higher School, but unfortunately died

soon after. Thus his manuscript is left as such, and has remained for the most part unpublished. It is still preserved at the Botanical Institute of the Science College of the Tokyo Imperial University with the title of " Hepaticae of Hakone, Idsu, Nikko, Tokyo and its vicinity (1887) ". I went to the Botanical Institute of the Tokyo Imperial University and stayed there for four weeks at the end of 1927, and due to the generosity of Prof. T. NAKAI, I was able to copy TSUGE's manuscript and examine the specimens of Hepaticae.

This study was undertaken originally at the suggestion of Prof. T. INUI of the Hiroshima Normal College, during my stay at Hiroshima in 1922, and has continued up to the present day. The author wishes here to acknowledge his obligation to Prof. M. TAHARA and to Assistant Prof. A. KIMURA of our University, for the valuable suggestions and criticism given in various ways, and also to Prof. B. HAYATA, Prof. T. NAKAI of the Tokyo Imperial University, Prof. T. INUI of the Hiroshima Normal College and Prof. S. OKAMURA of the Keio University for their kind advice and encouragement. Thanks are also due to Lecturer T. YOSHINAGA of the Kôchi Higher School and to Government Official Y. SHIMADA of Shinchikushû-chô in Formosa, who kindly placed at my disposal many valuable specimens collected mostly by themselves.

BIBLIOGRAPHY.

Only the more important papers relating to Japanese Hepaticae are mentioned in the following list. It does not include, therefore, local catalogues in which species are merely enumerated.

1) ANDREAS, J., 1899. Über den Bau der Wand und die Öffnungsweise des Lebermoossporogons. Flora, Bd. 86, pp. 161-213, Tab. XII.

2) BERGDOLT, E., 1926. Untersuchungen über Marchantiaceen. Botanische Abhandlungen, Heft 10. Jena.

3) BESCHERELLE, E., 1894. Enumération des Hépatiques récoltées par M l'abbé FAURIE au Japon et déterminées par M. STEPHANI. Rev Bryol., Vol. 21, pp. 25-27.

4) CAMPBELL, D. H., 1918. The Structure and Development of Mosses and Ferns. Ed. 3. New York.

5) CAMPBELL, D. H., 1918. Studies on some East Indian Hepaticae. Ann. Bot., Vol. 32, pp. 319-338, Pl. VIII-IX & 10 Textfigs.

6) CAMPBELL, D. H., 1920. Studies in some East Indian Hepaticae. Ann. Bot., Vol. 34, pp. 1-12, Pl. I & 6 Textfigs.

7) CARRINGTON, B., 1875. Monograph of the British Hepaticae. London.

8) COKER, W. C., 1903. Selected Notes. II.-Liverworts. Bot. Gaz., Vol. 36, pp. 225-230, 5 Figs.

9) COOK, J. C. M., 1907. The Hawaiian Hepaticae of the tribe Trigonantheae. Trans. Conn. Acad., Vol. 12, pp. 1-44, Pl. I-XV.

10) EVANS, A. W., 1892. An arrangement of Genera of Hepaticae. Trans. Conn. Acad., Vol. 8. p. 262.

11) EVANS, A. W., 1896. Notes on the North American species of *Plagiochila*. Bot. Gaz., Vol. 21, pp. 185-194, Pl. XV-XVI.

12) EVANS, A. W., 1897. Revision of the North American species of *Frullania*, a genus of Hepaticae. Trans. Conn. Acad., Vol. 10, 39 pp. 15 Pl.

13) EVANS, A. W., 1900. The Hawaiian Hepaticae of the tribe Jubuloideae. Trans. Conn. Acad., Vol. 10, pp. 387-462. 16. Pl.

14) EVANS, A. W., 1902. The Lejeuneae of the United States and Canada. Mem. Torrey Bot. Club, Vol. 8, pp. 113-183. Pl. XVI-XXII.

15) EVANS, A. W., 1902. Hepaticae of Puerto Rico. I. Bull. Torrey Bot. Club, Vol. 29, pp. 495-510. Pl. XXIII-XXV.

16) EVANS, A. W., 1903. Hepaticae of Puerto Rico. II. Bull. Torrey Bot. Club, Vol. 30, pp. 19-41. Pl. I-VI.

17) EVANS, A. W., 1903. Hepaticae of Puerto Rico. III. Bull. Torrey Bot. Club, Vol. 30, pp. 544-563. Pl. XX-XXII.

18) EVANS, A. W., 1904. Notes on New England Hepaticae,-II. Rhodora, Vol. 6, pp. 165-191. Pl. LVII.

19) EVANS, A. W., 1905. A remarkable *Ptilidium* from Japan. Rev. Bryol., Vol. 32, pp. 57-60.

20) EVANS, A. W., 1905. Notes on New England Hepaticae,-III. Rhodora, Vol. 7, pp. 52-58.

21) EVANS, A. W., 1906. Hepaticae of Puerto Rico. VI. Bull. Torrey Club, Vol. 33, pp. 1-25. Pl. I-III.

22) EVANS, A. W., 1906. Notes on New England Hepaticae,-IV. Rhodora, Vol. 8, pp. 34-45.

23) EVANS, A. W., 1906. Notes on Japanese Hepaticae. Proc. Wash. Acad. Sci., Vol. VIII, pp. 141-166. Pl. VI-VIII.

24) EVANS, A. W., 1907. Hepaticae of Puerto Rico. VII. Bull. Torrey Bot. Club, Vol. 34, pp. 1-34. Pl. I-IV.

25) EVANS, A. W., 1907. Notes on New England Hepaticae,-V. Rhodora, Vol. 9, pp. 56-73. Pl. 73.

26) EVANS, A. W., 1907. The Genus *Calypogeia* and its Type Species. Bryologist, Vol. 10, pp. 24-30.

27) EVANS, A. W., 1907. *Leucolejeunea*, A New Genus of Hepaticae. Torreya, Vol. 7, pp. 225-229.

28) EVANS, A. W., 1908. Hepaticae of Puerto Rico. IX. Bull. Torrey Bot. Club, Vol. 35, pp. 155-179. Pl. VI-VIII.

29) EVANS, A. W., 1908.   Notes on New England Hepaticae,·VI. Rhodora, Vol. 10, pp. 185–193.

30) EVANS, A. W., 1909.   Notes on New England Hepaticae,·VII. Rhodora, Vol. 11, pp. 185-195.

31) EVANS, A. W., 1910.   Notes on New England Hepaticae,·VIII. Rhodora, Vol. 12, pp. 195-204.

32) EVANS, A. W., 1911.   Notes on North American Hepaticae. II. Bryologist, Vol. 10, pp. 84–88.

33) EVANS, A. W., 1911.   Hepaticae of Puerto Rico. X. Bull. Torrey Bot. Club, Vol. 38, pp. 251-286. Pl. XI-XII.

34) EVANS, A. W., 1912.   Notes on New England Hepaticae,·IX. Rhodora, Vol. 14, pp. 1–18.

35) EVANS, A. W., 1912.   Notes on New England Hepaticae,·X. Rhodora, Vol. 14, pp. 209-225.

36) EVANS, A. W., 1913.   Notes on North American Hepaticae. IV. Bryologist, Vol. 16, pp. 50-55.

37) EVANS, A. W., 1914.   Notes on New England Hepaticae,·XI. Rhodora, Vol. 16, pp. 62-66.

38) EVANS, A. W., 1914.   Notes on North American Hepaticae. V. Bryologist, Vol. 17, pp. 87-92.

39) EVANS, A. W., 1915.   The genus *Plagiochasma* and its North American species. Bull. Torrey Bot. Club, Vol. 42, pp. 259–308. Textfigs. 1–8.

40) EVANS, A. W., 1915.   Notes on New England Hepaticae,·XII. Rhodora, Vol. 17, pp. 107–120.

41) EVANS, A. W., 1916.   Notes on New England Hepaticae,·XIII. Rhodora, Vol. 18, pp. 74–120. Pl. 120.

42) EVANS, A. W., 1917.   Notes on North American Hepaticae, VII. Bryologist, Vol. 20, pp. 17–28. Pl. II.

43) EVANS, A. W., 1917.   The American Species of *Marchantia*. Trans. Conn. Acad., Vol. 21, pp. 201–313. Textfigs. 1–20.

44) EVANS, A. W., 1917.   Notes on New England Hepaticae,·XIV. Rhodora, Vol. 19, pp. 263-272.

45) EVANS, A. W., 1917.   Notes on the genus *Herberta*, with a revision of the species known from Europe, Canada and the United States. Bull. Torrey Bot. Club, Vol. 44, pp. 191-222. Pl. VIII & Textfigs. 1–29.

46) EVANS, A. W., 1919.   Notes on North American Hepaticae, VIII. Bryologist, Vol. 22, pp. 54–73. Pl. II.

47) EVANS, A. W., 1919.   A taxonomic study of *Dumortiera*. Bull. Torrey Bot. Club, Vol. 46, pp. 167–182.

48) EVANS, A. W., 1919.   Notes on New England Hepaticae,·IV. Rhodora, Vol. 21, pp. 149-169. Pl. 126.

49) EVANS, A. W., 1923.   Corsiniaceae, Targioniaceae, Sauteriaceae, Rebouliaceae, Marchantiaceae. North Amer. Flora, Vol. 14, pp. 29–66.

50) FALCONER, H., 1851.   On *Athalamia*, a new Genus of Marchantieae. Trans. Linn. Soc., Vol. 20, Part 3, p. 397–398. Tab. XIX.

51) GIBBS, S. S., 1920. The genus *Calobryum*. Jour. Bot., Vol. 58, p. 275.

52) GOEBEL, K., 1890. Morphologische und biologische Studien. Ann. Jardin Bot. Buitenzorg, Vol. 9, pp. 11–15. Tab. II.

53) GOEBEL, K., 1913. Organographie der Pflanzen. Erster Teil. Allgemeine Organographie. 2-Auflage, Jena.

54) GOEBEL, K , 1915–1918. Organographie der Pflanzen. Zweiter Teil. Spezielle Organographie. 2-Auflage, Jena.

55) GOTTSCHE, C. M., LINDENBERG, J. B. G. et NEES AB ESENBECK, C. G., 1844–1847. Synopsis Hepaticarum. Hamburg.

56) HAYATA, B., 1928. On some of the most remarkable species of the Japanese Hepaticeae. Bot. Magaz. Tokyo, Vol. 42, pp. 181–190 (Japanese).

57) HERZOG, T., 1925. Anatomie der Lebermoose, in LINSBAUER's Handbuch der Pflanzenanatomie. Bd. VII/1, pp. 1–112. Berlin.

58) HORIKAWA, Y., 1928. On *Cavicularia densa* STEPHANI. Sci. Report Tôhoku Imp. Univ. 4th Series, Vol. 3, pp. 259–264. Pl. II.

59) HOWE, M. A., 1923. Ricciaceae. North Amer. Flora, Vol. 14, pp. 11–27.

60) HUSNOT, T., 1920. Hepaticologia Gallica. Ed. 2. Cahan par Athis (Orne).

61) IISHIBA, E., 1909. On the Japanese *Frullania*. Bot. Magaz. Tokyo, Vol. 23, pp. 363–364 (Japanese).

62) IISHIBA, E., 1912. Review on the *Frullania* in Japan. Bot. Magaz. Tokyo, Vol. 26, pp. 31–37 (Japanese).

63) INOUE, T., 1894. Hepaticeae of Tosa. I. Bot. Magaz. Tokyo, Vol. 8, pp. 291–293 (Japanese).

64) INOUE, T., 1895. Hepaticeae of Tosa. II. Bot. Magaz. Tokyo, Vol. 9, pp. 134–135 (Japanese).

65) JACK, J. B., 1886. Monographie der Lebermoosgattung *Physiotium*. Hedwigia, pp. 49–87. 10 Taf.

66) JACK, J. B., 1895. Beiträge zur Kenntniss der *Pellia*-Arten. Flora, Bd. 81, pp. 1–16. Taf. I.

67) LEITGEB, H., 1974. Untersuchungen über die Lebermoose. I Heft. *Blasia pusilla*. Berlin.

68) LEITGEB, H., 1875. Untersuchungen über die Lebermoose. II Heft. Die Foliosen Jungermannieen. Berlin.

69) LEITGEB, H., 1877. Untersuchungen über die Lebermoose. III Heft. Die Frondosen Jungermannieen. Berlin.

70) LEITGEB, H., 1879. Untersuchungen über die Lebermoose. IV Heft. Die Riccieen. Graz.

71) LEITGEB, H., 1879. Untersuchungen über die Lebermoose. V Heft. Die Anthoceroteen. Graz.

72) LEITGEB, H., 1881. Untersuchungen über die Lebermoose. VI Heft. Die Marchantieen und allgemeine Bemerkungen über Lebermoose. Graz.

73) LINDBERG, S. O., 1872. Contributio ad Floram Cryptogaman Asiae borealiorientalis (Musci Japonici, collecti a clar C. MAXIMOVICZ). Acta Soc. Sc. Fenn., Vol. 10, pp. 223–280.

74) LINDBERG, S. O., 1875. Hedwigia, p. 139.

75) LINDBERG, S. O., 1884. *Sandea* et *Myriorhnchus*, nova Hepaticarum genera. Acta Soc. pro Fauna et Flora Fenn., Vol. 2, No. 5, pp. 1-9.

76) LORCH, W., 1926. Lebermoose, in LINDAU's Kryptogamenflora, Bd. 6, pp. 1-232. 2-Aufl. Berlin.

77) MACVICAR, S. M., 1926. The Student's Handbook of British Hepatics. Ed. 2. Eastbourne.

78) MAKINO, T., 1896. Hepaticae of Prov. Tosa and of Tokyo. Bot. Magaz. Tokyo, Vol. 10, p. 209 (Japanese).

79) MASSALONGO, C., 1897. Hepaticae in provincia Schen-si, Chinae interioris, a rev. P. JOS. GIRALDI collectae. Mem. Acad. Verona, Vol. 73, 3 Ser., pp. 5-63. 14 Taf.

80) MEYLAN, C., 1924. Les Hépatiques de la Suisse, in Beiträge zur Kryptogamenflora der Schweiz, Bd. 6, Heft 1. Zürich.

81) MICHELI, P. A., 1729. Nova plantarum genera.

82) MIQUEL, F. A. G., 1866-1867. Musci et Hepaticae, determinavit cl. VAN DER SANDE LACOSTE. MIQUEL's Prolusio Flor. Japon., p. 373. & MIQUEL's Ann. Mus Bot. Lugduno-Batavi., Vol. 3, (1867), p. 209.

83) MIQUEL, F. A. G., 1866-1867. Conspectus Florae Japonicae. MIQUEL's Prolusio Flor. Japon., Accedunt Fabulae II, (Hepaticae), p. 391.

84) MIQUEL, F. A. G., 1870. Flora Japonica, in Catalogus Musei Botanici Lugduno-Batavi, Pars I, (Hepaticae)., pp. 135-137.

85) MITTEN, W., 1865. On some Species of Musci and Hepaticae, additional to the Floras of Japan and the coast of China. Jour. Linn. Soc., Bot., Vol. 8, (Hepaticae) pp. 148-158.

86) MITTEN, W., 1891. An Enumeration of all species of Musci and Hepaticae recorded from Japan. Trans. Linn. Soc., 2nd Series, Vol. 3, pp. 153-206. Pl. LVIII.

87) MIYAKE, K., 1898. A New Genus of Hepaticae. Bot. Magaz. Tokyo, Vol. 12, pp. 85-86 (Japanese).

88) MIYAKE, K., 1899. *Makinoa*, a New Genus of Hepaticae. Bot. Magaz. Tokyo, Vol. 13, pp. 21-24. Pl. III. & in Hedwigia, Bd. 38, (1899), p. 201. I Taf. & MATSUMURA et MIYOSHI's Cryptogamae Japonicae iconibus illustratae. Vol. 1, No. 7, (1899), Pl. XXXV, (Japanese).

89) MIYAKE, K., 1899. *Marchantia polymorpha* L. et *Conocephalum conicum* NECKER, in MATSUMURA et MIYOSHI's Cryptogamae Japonicae iconibus illustratae. Vol. 1, No. 2 et No. 4, 2 Pl., (Japanese).

90) MIYAKE, K., 1899. On the sexual organs of *Cavicularia*. Bot. Magaz. Tokyo, Vol. 13, p. 270 (Japanese).

91) MOLISCH, H., 1925. Über die Symbiose der beiden Lebermoose *Blasia pusilla* L. und *Cavicularia densa* ST. mit *Nostoc*. Sci. Report Tôhoku Imp. Univ., 4th Series, Vol. 1, pp. 169-188. Taf. I-II.

92) MÜLLER, K, 1903. Neue und kritische Lebermoose. Bull Herb. Boissier, pp. 33-44. Taf.

93) MULLER, K., 1905. Monographie der Lebermoosgattung *Scapania* DUM. Nova Acta Acad. Caes. Leop.-Carol., Tom. LXXXIII, pp. 1-312. Taf. I LII.

94) MÜLLER, K., 1905-1911. Die Lebermoose Deutschlands, Oesterreichs u. d.

Schweiz, in RABENHORST's Kryptogamen-Flora, 2-Aufl., Bd. VI, Abt. 1, Leipzig.

95) MÜLLER, K., 1912-1916.   Die Lebermoose Deutschlands, Oesterreichs u. d.
Schweiz, in RABENHORST's Kryptogamen-Flora, 2-Aufl., Bd. VI, Abt. 2, Leipzig.

96) NEES VON ESENBECK, G. G., 1833-1338.   Naturgeschichte der europäischen
Lebermoose. 4 Bde. Berlin.

97) OKAMURA, S., 1911.   Neue Beiträge zur Moosflora Japans, I. Bot. Magaz. Tokyo,
Vol. 25, pp. 33-34.

98) OKAMURA, S., 1911.   Neue Beiträge zur Moosflora Japans. II. Bot. Magaz. Tokyo,
Vol. 25, p. 65.

99) OKAMURA, S., 1911.   Neue Beiträge zur Moosflora Japans. III. Bot. Magaz.
Tokyo, Vol. 25, pp. 159-162. Taf. V.

100) OKAMURA, S., 1914.   Über einige Arten von Bryophyten aus gewissen Seeboden
in Japan. Bot. Magaz. Tokyo, Vol. 28, pp. 407-413 (Japanese).

101) OKAMURA, S., 1915.   Über einige Arten von Bryophyten aus gewissen Seeboden
in Japan, (2-Berichte). Bot. Magaz. Tokyo, Vol. 29, pp. 334-336. Fig. 1 (Japanese).

102) OKAMURA, S., 1915.   Contributiones novae ad Floram Bryophyton Japonicam.
Pars prima. Jour. Coll. Sci. Tokyo Imp. Univ., Vol. 36, Artic. 7, (Hepaticae, pp.
1-3, Pl. I).

103) OKAMURA, S., 1916.   Contributiones novae ad Floram Bryophyton Japonicam.
Pars secunda. Jour. Coll. Sci. Tokyo Imp. Univ., Vol. 38, Artic. 4, (Hepaticae,
pp. 1-3. Fig. 1).

104) OKAMURA, S., 1925.   Hepaticae, in MAKINO's Nippon Shokubutshu Dzukwan.
(Japanese). Tokyo.

105) SANDE-LACOSTE, C. M. VAN DER, 1856.   Synopsis Hepaticarum Javanicarum
adjectis quibusdam speciebus Hepaticarum novis extra-Javanicis. Verh. der Koninkl.
Akad. V., Tab. I-XXII, 112 pp.

106) SANDE-LACOSTE, C. M. VAN DER, 1863-1864.   Hepaticae. Jungermaniaceae Archi-
pelagi Indici, adjectis quibusdam speciebus Japonicis. Ann. Musei Bot. Lugduno-
Batavi, Vol. 1, pp. 287-314.

107) SANDE-LACOSTE, C. M. VAN DER, 1867.   Musci et Hepaticae. Ann. Musci Bot.
Lugduno-Batavi, Vol. 3, p. 209.

108) SAWADA, K., 1909.   *Frullania* in Japan. Bot. Magaz. Tokyo, Vol. 23, pp. 178-
183. 1 Pl. (Japanese).

109) SCHADE, A., 1923.   Bemerkungen zur Warnstorfs Arbeit über „ die europäischen
Artgruppen der Gattung *Calypogeia* RADDI (1820)". Hedwigia, Bd. 65, pp. 1-10.

110) SCHIFFNER, V., 1893.   Hepaticae, in ENGLER u. PRANTL's Natürl. Pflanzen-Fam.,
I Teil, 3 Abt., 1 Hälfte. Leipzig.

111) SCHIFFNER, V., 1893.   Über exotische Hepaticae. Nova Acta Acad. Caes., Vol.
60, pp. 219-316. Pl. VI-XIX.

112) SCHIFFNER, V., 1896.   *Wiesnerella*, eine neue Gattung der Marchantiaceen.
Oesterr. Bot. Zeitsch., (1896), Nr. 3, 6 pp. 1 Taf.

113) SCHIFFNER, V., 1897.   Revision der Gattungen *Omphalanthus* und *Lejeunea* im
Herbarium des Berliner Museums. Bot. Jahrb., Bd. 23, pp. 578-600. Taf. XV.

114) SCHIFFNER, V., 1898.   Conspectus Hepaticarum Archipelagi Indici. Herausgeg.
vom Bot. Garten in Buitenzorg. Batavia.

115) SCHIFFNER, V., 1898. Expositio Plantarum in Itinere Suo Indico Annis 1893–1894 Suscepto Collectarum. Ser. Prima. Hepaticarum Partem Continens. Denkschr. d. Mathem.-Naturw., Bd. 67, 51 pp.

116) SCHIFFNER, V., 1899. Expositio Plantarum in Itinere Suo Indico Annis 1893–1894 Suscepto Collectarum. Ser. Secunda. Hepaticarum Partem Alteram Continens. Denkschr. d. Mathem.-Naturw., Vol. 70, pp. 155–218.

117) SCHIFFNER, V., 1899. Über einige Hepaticae aus Japan. Oesterr. Bot. Zeitsch., Bd. 49, pp. 385–395.

118) SCHIFFNER, V., 1900. Die Hepaticae der Flora von Buitenzorg. Erster Band. pp. 1–220.

119) SCHIFFNER, V., 1900. Kritische Studien über *Jungermania sinuata* DICKS. und *Aneura pinnatifida* N. AB E., sowie über *Riccardia major* S. O. LINDB. und *Riccardia incurvata* S. O. LINDB. Lotos. (1900) Nr. 8.

120) SCHIFFNER, V., 1900. WARBURG; Monsunia, Vol. 1. p. 41.

121) SCHIFFNER, V., 1901. Untersuchungen über *Morckia Flotowiana* und über das Verhältnis der Gattungen *Morckia* GOTT. und *Calycularia* MITT. zu einander. Oesterr. Bot. Zeitsch., (1901), Nr. 2.

122) SCHIFFNER, V., 1901. Einige Untersuchungen über die Gattung *Makinoa*. Oesterr. Bot. Zeitsch., (1901), Nr. 3, pp. 82–89. Taf. I.

123) SCHIFFNER, V., 1903. Studien über kritische Arten der Gattungen *Gymnomitrium* und *Marsupella*. Oesterr. Bot. Zeitsch., (1903), Nr. 3, 31 pp. Taf. II-IV.

124) SCHIFFNER, V., 1904. Über die Variabilität von *Nardia crenulata* und *N. hyalina*. Verh. Zool. Bot. Ges. in Wien, (1904), pp. 410–422.

125) SCHIFFNER, V., 1904. Über *Dumortiera*. Hedwigia, Bd. 43, pp. 428–429.

126) SCHIFFNER, V., 1907. Morphologische und biologische Untersuchungen über die Gattungen *Grimaldia* und *Neesiella*. Hedwigia, Bd. 47, pp. 306–320. Taf. VIII.

127) SCHIFFNER, V., 1910. Untersuchungen über Amphigastrial-Antheridien und über den Bau der Andröcien der Ptilidioideen. Hedwigia, Bd. 50, pp. 146–162.

128) SCHIFFNER, V., 1910. Motion au Congrés international de Botanique de Bruxelles 1910, relative à la Nomenclature des Hépatiques. Vienne.

129) SCHIFFNER, V., 1912. Kritik der europäischen Formen der Gattung *Chiloscyphus* auf phylogenetischer Grundlage. Beih. Bot. Centralbl., Bd. 29 II. Abt., pp. 74–116. 2 Taf.

130) SCHIFFNER, V., 1914. Hepaticae, in Die Süsswasser-Flora Deutschlands, Oesterreichs und der Schweiz.

131) SCHIFFNER, V., 1914. *Cephalozia*-Studien. Hedwigia, Bd. 54, pp. 311–327. Taf. XI u. 1 Textfig.

132) SPRUCE, R., 1882. On *Cephalozia*, a genus of Hepaticae, its subgenera and some allied genera. Malton.

133) SPRUCE, R., 1885. Hepaticae of the Amazon and of the Andes of Peru and Ecuador. Trans. Bot. Soc. Edinb., Vol. 15, pp. 1–590. Pl I–XXII.

134) STEPHANI, F., 1879. Deutschlands Jungermannien in Abbildungen nach der Natur. Bot. Verein in Landschut, Vol. 7, pp. 93–164. 31 Taf.

135) STEPHANI, F., 1883. Einige neue Lebermoose. Hedwigia, (1883), Heft. 4, pp. 49–52.

136) STEPHANI, F., 1884. Die Gattung *Radula*. Hedwigia, Bd. 23, pp. 145-160.

137) STEPHANI, F., 1890. Die Gattung *Lejeunea* in Herbarium Lindenberg. Hedwigia, (1890), Heft 1, 2 u. 3, pp. 1-23; 68-99; 133-142.

138) STEPHANI, F., 1893. Hepaticarum species novae. III. Hedwigia, Bd. 32, Heft 4, pp. 204-214.

139) STEPHANI, F., 1894. Hepaticarum species novae. V. Hedwigia, Bd. 33, Heft 1, pp. 1-10.

140) STEPHANI, V., 1894. Eine neue Lebermoos-Gattung. Oesterr. Bot. Zeitsch. Bd. 44, pp. 1-5. Taf. I.

141) STEPHANI, V., 1895. Hepaticarum species novae. VII. Hedwigia, Bd. 34, Heft 1 u. 2, pp. 43-48; 49-65.

142) STEPHANI, V., 1896. Hepaticarum species novae. IX. Hedwigia, Bd. 35, pp. 73-140.

143) STEPHANI, V., 1897. Hepaticae Japonicae. Bull. Herbier Boissier, Vol. 5, pp. 76-108.

144) STEPHANI, V., 1898-1900. Species Hepaticarum. Vol. I. Sep.-Abdr. aus dem Bull. et Mém. Herbier Boissier. Genève et Bale.

145) STEPHANI, V., 1901-1906. Species Hepaticarum. Vol. II. Sep.-Abdr. aus dem Bull. Herbier Boissier. Genève et Bale.

146) STEPHANI, V., 1904. Hepaticarum species novae. X. Hedwigia, Bd. 44, pp. 14-15.

147) STEPHANI, V., 1906-1909. Species Hepaticarum. Vol. III. Sep.-Abdr. aus dem Bull. Herbier Boissier. Genève et Bale.

148) STEPHANI, V., 1909-1912. Species Hepaticarum, Vol. IV. Sep.-Abdr. aus dem Bull. Herbier Boissier. Genève et Bale.

149) STEPHANI, V., 1912-1917. Species Hepaticarum. Vol. V. Sep.-Abdr. aus dem Bull. Herbier Boissier. Genève et Bale.

150) STEPHANI, V., 1917-1824. Species Hepaticarum. Vol. VI. Université de Genève et Herbier Boissier. Genève.

151) SULLIVANT, W. S., 1856. Hepaticae, in GRAY's Manual of Botany, Ed. 2. pp. 682(82)-702(102). Pl. VI-VIII.

152) TAYLOR, T., 1846. New Hepaticae. Lond. Jour. Bot., Vol. 5, pp. 258-284; 365-417.

153) THUNBERG, C. P., 1784. Flora Japonica, etc., Hepaticae p. 343., Leipzig.

154) TILDEN, J. E., 1894. On the morphology of hepatic elaters, with special reference to branching elaters of *Conocephalus conicus*. Minnesota Bot. Stud., Bull. No. 9, pp. 43-52. Minneapolis.

155) TSUGE, C., 1887. Hepaticae of Hakone, Idsu, Nikko, Tokyo and its vicinity. (unpublished) Mss. In Botanical Institute, Faculty of Science of Tokyo Imp. University.

156) TSUGE, C., 1887. *Marchantia polymorpha* L., Bot. Magaz. Tokyo, Vol. 1, pp. 151-154. Pl. XIX, (Japanese).

157) TSUGE, C., 1887. Marchantiaceae. Bot. Magaz. Tokyo, Vol. 1, pp. 169-172, (Japanese).

158) TSUGE, C., 1890. Tairui Ippan (On Hepaticae). Bot. Magaz. Tokyo, Vol. 4. pp. 208-213. Pl. VII, (Japanese).

159) Tsuge, C., 1890. Tairui Ippan (On Hepaticae). Bot. Magaz. Tokyo. Vol. 4, pp. 241–242. Pl. VIII, (Japanese).

160) Underwood, L. M., 1888. Some undescribed Hepaticae from California. Bot. Gaz.. Vol. 14, pp. 112–114. Pl. III–VI.

161) Underwood, L. M., 1890. Hepaticae, in Gray's Manual of Botany, 6th Ed. pp. 702–732. Pl. 22–25. New York.

162) Underwood, L. M., 1892. A preliminary comparison of the Hepatic Flora of Boreal and Sub-boreal Regions. Bot. Gaz., Vol. 17, pp. 305–312.

163) Underwood, L. M., 1895. Hepaticae, in Systematic Botany of North America, Vol. 9, Part I.

164) Voigt, A., 1879. Ein Beitrag zur vergleichenden Anatomie der Marchantiaceen. Bot. Zeit., Bd. 37, pp. 729–743; 745–759. Pl. IX.

165) Warnstorf, C., 1903. Leber- und Torfmoose in Kryptogamen-Flora der Mark Brandenburg.

166) Warnstorf, C., 1920. Über einige Arten aus der Gattung *Calypogeia* Raddi sensu Nees. Hedwigia, Bd. 62, pp. 1–11.

167) Warnstorf, C., 1921. Die Unterfamilie der Scapanioideen. (Spruce 1885). Hedwigia, Bd. 63, pp. 58–116.

168) Yoshinaga, T., 1901. On some newly discovered Hepaticae from Tosa and Iyo. Bot. Magaz. Tokyo, Vol. 15, pp. 91–94, (Japanese).

169) Yoshinaga, T., 1903. On some new Hepaticae from Tosa and Nikko. Bot. Magaz. Tokyo, Vol. 17, pp. 37–39, (Japanese).

Genus: **REBOULIA** Raddi (1818) emend. Nees (1846)

Reboulia hemisphaerica (L.) Raddi
(Text-Fig. 1.)

Reboulia longipes Sande-Lac. in Miquel, Flora Japonica, p. 379 (1870).

Reboulia hemisphaerica (L.) Raddi; Tsuge, Bot. Magaz. Tokyo, Vol. I, p. 172 (1887); Schiffner, in Engler u. Prantl, Natürl. Pflanzenfam., Teil I, Abt. 3, p. 31 (1893); Stephani, Spec. Hepat., Vol. I, pp. 790–791 (1898); Müller, in Rabenhorst's Kryptogamen-Flora, Bd. 6, Abt. I, pp. 255–259 (1907), Okamura, in Makino, Nipp. Shokub. Dzukwan, p. 1253 (1925); Macvicar, Student's Handb. Brit. Hepat., pp. 35–37 (1926).

Dioicous or monoicous. Mesophyte. Growing in small rosettes or slightly extended layers. Thallus 20–40 mm. long and 5–7 mm. broad, furcate, firm and leathery, green to light green, slightly shiny, often more or less pigmented with purple on lower surface and margin, the margin somewhat ascending and crenulated. Dorsal epidermis composed of 4–6-angled cells with large trigones, mostly 14.5–23.5 μ wide (averaging about 15 μ) and 27.5–60 μ long (averaging
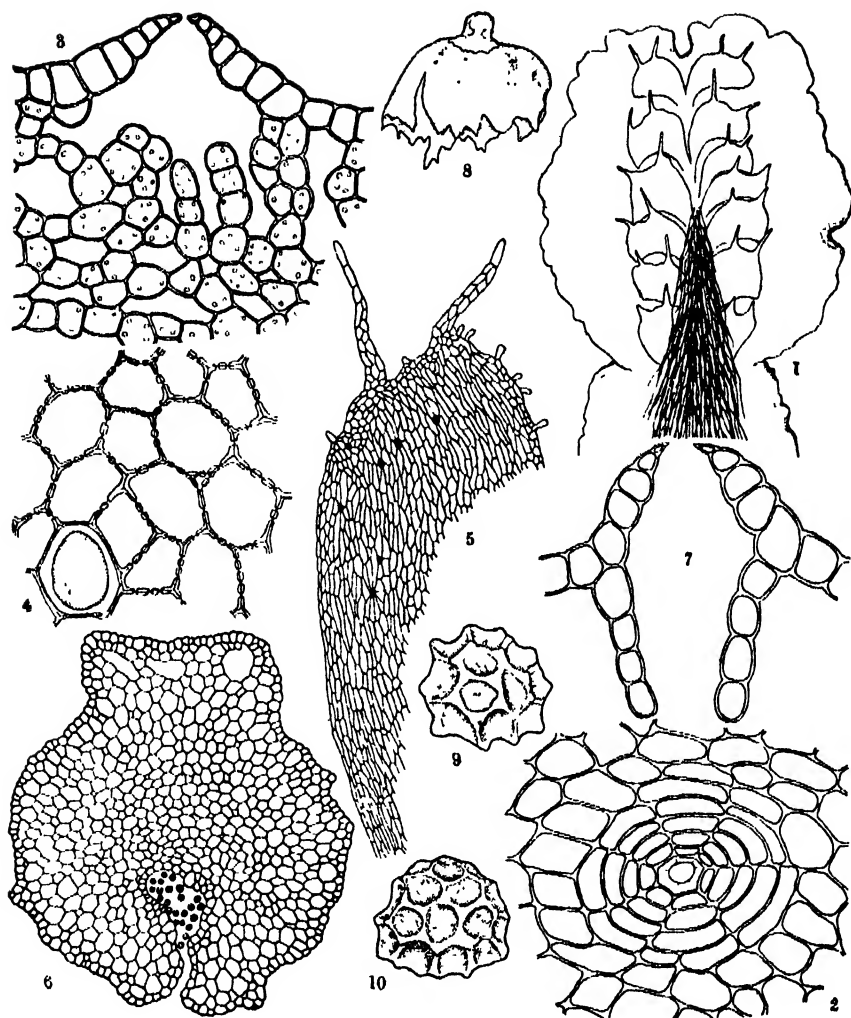
Text-Fig. 1. *Reboulia hemisphaerica* (L.) RADDI

1. Anterior part of thallus, postical view, ×8. 2. Epidermal pore of thallus, surface view, ×233. 3. Cross-section of thallus, showing a simple pore, ×233. 4. Cross-section of ventral compact tissue, showing a oil-cell and simple pits, ×300. 5. Ventral scale with two appendages, ×38. 6. ♀ receptacle, in cross-section near base, ×75. 7. Compound pore in cross-section, from ♀ receptacle, ×233. 8. Capsule after splitting, showing the state of being covered by a calyptra at the base, ×233.

about 39 μ). Pores (with their surrounding cells) simple, elevated, mostly 118–131 μ long and 65.5–106 μ wide, with 5–6 concentric rings, each ring usually being composed of 6–8 cells with thickened angles. Rhizoids numerous and colourless, 12.7–19 μ wide, either smooth or tuberculate. Air-chambers strongly developed, their boundaries indistinct, occupying about 1/3 of thickness in the middle and almost all of it in the marginal portion. Compact ventral tissue mostly 17–20 cells thick in the median portion, the walls more or less thickened and showing distinct simple pits; oil cells distinct, scattered, gradually passing into the lamina ending in a 1-celled margin. Ventral scales imbricate, in one row on each side of the midrib, deeply pigmented with purple, obliquely lunate, with 2–3 linear acute appendages, 7–9 papillae at margin and many oil-cells. ♀ receptacle conical or hemispherical, green, divided to the middle into 3–5 obtuse lobes (mostly 4) which at first bended downwards, later becoming horizontal or revolute, each lobe with air-chambers and compound (barrel-shaped) pores. Involucres whitish, arising from the ventral margin of the receptacle-lobes, conchoidal and 2-valved along the ventral longitudinal line, each enclosing a single sporogonium which later half exserts. Capsule subglobose, very shortly pedicellate, greenish-black, irregularly dehiscing at the apex, the lower portion being left behind as a hemispherical cup containing spores and elaters. Capsule-wall of a single layer of cells, without annular thickenings, containing numerous chloroplasts. Peduncle of ♀ receptacle 20–60 mm. long, with assimilation-tissue, light brown below, surrounded by numerous, filamentous, 2–4 cells-rowed hairs on both lower and upper ends, with one ventral-furrow. Perianth absent. Spores 64–78 μ in diameter, brownish-yellow, rounded-tetrahedral, minutely verruculose, with a few large areolae and with broad (10 μ) crenulate margin. Elaters brownish-yellow, bent, gradually attenuate, mostly 341–394 μ long and 8.5 μ wide in maximum diameter, with two or three spirals. ♂ receptacle sessile, arising from behind the apex of a thallus-lobe, oval to semicircular, surrounded with small paleae. Gemmae absent.

Fr. March-May.

Hab. On rocks and on soil among rocks in rather dry places.

Loc.

Honshiu : Matsushima, prov. Rikuzen (Y. HORIKAWA, no. 778, July

1927) ; Sendai, prov. Rikuzen (Y. HORIKAWA, no. 1255, June 1928) ;
Nagaoka, prov. Echigo (M. JÛMI, no. 1218, June 1928) ; Nikko, prov.
Shimozuke (C. TSUGE, May 1887) ; Tokyo (C. TSUGE, 1887) ; ibidem
(K. MIYAKE, no. 114, 1897) ; Hakone, prov. Sagami (C. TSUGE, Apr.
1887) ; prov. Idsu (C. TSUGE, Apr. 1887) ; Hiroshima, prov. Aki (Y.
HORIKAWA, Apr. 1924) ; Insl. Miyajima, prov. Aki (Y. HORIKAWA. no.
151, March 1924 & no. 644, Apr. 1927).

Shikoku : Sakawa-machi, prov. Tosa (T. YOSHINAGA, no. 1244,
July 1928).

Kiushiu : Nankwan, prov. Higo (Y. HORIKAWA, no. 37, March
1923) ; Kawamura, prov. Higo (K. MAYEBARA, no. 22, March 1927) ;
Aida, prov. Higo (K. MAYEBARA, no. 2 & no. 14, Feb. 1927) ; Kônose,
prov. Higo (K. MAYEBARA, no. 48, March 1927) ; Aoshima-mura, prov.
Hiuga (Y. HORIKAWA, no. 360, Apr. 1927) ; Kagoshima, prov. Satsuma
(Y. HORIKAWA, no. 536, Apr. 1927).

Formosa : Urai, prov. Taihoku (J. SHIRAGA, July 1911) ; Kei-
chikurin, prov. Shinchiku (Y. SHIMADA, no. 16, Feb. 1928) ; Kôzanshô,
prov. Shinchiku (Y. SHIMADA, no. 9, Dec. 1927) ; Kômôkwan, prov.
Shinchiku (Y. SHIMADA, no. 3, Nov. 1927) ; Shinchikugai, prov. Shin-
chiku (Y. SHIMADA, no. 21, March 1928).

Distrib. Europe, Himalaya, Java, Caucasus, America, Africa,
Australia and New Zealand.

Remarks. The genus is monotypic but very polymorphic.

Genus : **MAKINOA** MIYAKE (1899)

Makinoa MIYAKE, Bot. Magaz. Tokyo, Vol. XIII, p. 21 (1899) & Hedwigia, Bd.
XXXVIII, p. 201 (1899) & in MATSUMURA et MIYOSHI, Cryptog. Japon. Vol. I, no. 7,
Pl. XXXV. (1899); STEPHANI, Spec. Hepat., Vol. I, p. 361 (1900); SCHIFFNER, Oesterr.
Bot. Zeitsch. pp. 82-89 (1901).

Thallus large, fleshy, undulate and crispate at margin, dichotomously
branched with a broad midrib, passing gradually into unistratose
margin. Rhizoids numerous from the ventral side of midrib. Under-
leaves filamentous in several rows on each side of the midrib. Arche-
gonia grouped 15-18 in a depression, covered by an undulate and
ciliate involucre opening in front. Perianth absent. Calyptra free and
large, bearing numerous unfertilized archegonia (thalamogenous) on

the surface throughout. Capsule long-pedicellate, oblong-cylindrical, dehiscing almost to the base along one longitudinal line, the wall with two or three layers of cells, with distinct trigones; the outermost layer thicker, with many scalariform thickenings on radial walls, the inner layer thinner and prosenchymatous. Elater-bearers persistent as erect tufts on the apex of the valve. Spores rather small, 1-celled. Elaters very long, stout, arranged parallel to the long axis of capsule, spiral only in the middle portion, about one-third of the entire length, coalescing along a longitudinal line, tapering gradually to slender extremities. ♂ plant separate; antheridia not scattered but grouped 20–30 in a crescent-shaped receptacle and arranged regularly in the hinder part of a depression, the oldest in hindpart, the youngest in forepart, spherical with a short pedicel, immersed singly in each large alveolus, covered with a dome-shaped layer, the wall-cells thick and cubic; spermatozoids large.

Makinoa crispata (STEPH.) MIYAKE
(Pl. VI & VII.)

Pellia crispata STEPHANI, Bull. Herb. Boissier, Vol. V, p. 103 (1897).

Makinoa crispata (STEPH.) MIYAKE, loc. cit. (1899); OKAMURA, in MAKINO, Nipp. Shokub. Dzukwan, p. 1259 (1925); HAYATA, Bot. Magaz. Tokyo, Vol. XLII, p. 188 (1928).

Dioicous. Mesophyte. Growing in extended layers, dark to brownish-yellow or pale green. Thallus 65–70 mm. long or more and 11–15 mm. broad, tender, furcate, margin undulate and usually greatly crispate, channelled above the dark-green midrib, in section 10–12 cells thick in the middle, the horizontal, longitudinal cell-walls thickened, outer cells smaller than the inner, broadly concave above, convex below, gradually passing into the 1-celled layer of lamina. Rhizoids numerous from the midrib, brown, 10.5–21 μ in diameter. Underleaves filamentous, of a single series of 3–5 cells in 2–4 rows on each side of the midrib, apex-cell club-shaped and somewhat brown in colour, and evanescent. Involucre reduced to a semi-cylindrical scale-like flap on the posterior side of the cavity, dentated and undulated at the free margin, persistent for many years. Calyptra much-exserted, cylindrical, obliquely erect, 6–9 mm. long and 2–2.15 mm. in diameter, 7–8 cells thick above, 9–10 cells thick below, brownish-yellow but pale green

when young, roughened with many longer unfertilized scattered archegonia, the mouth usually irregularly three-lobed, the lobes ciliate. Capsule oblong-cylindrical, blackish-brown, shiny, 3–4 mm. long and 1.5 mm. in diameter, dehiscing to the base usually on one longitudinal line, sometimes dehiscing incompletely by 2 valves, the wall consisting of two or three layers; the outermost layer, one cell in thickness, with brownish-yellow scalariform thickenings in the radial walls, 42.5–53 μ in radial diameter and 30–42.5 μ in tangential diameter; the inner layer, one or two cells in thickness, strongly thickened at the corner, prosenchymatous mostly with pointed ends, 12.7–19 μ in radial diameter and 17–27.7 μ in tangential diameter, therefore the ratio in thickness of the inner· to the outermost layer averaging 1 : 3 in radial diameter, 10 : 17 in tangential diameter. Elater-bearers numerous, apical, attached to the apex of capsule. Pedicel 55–60 mm. long and 1.08–1.17 mm. in diameter, hyaline but pale green when young. ♀ inflorescence solitary or geminate in a depression on the dorsal surface of thallus, covered by an involucre; archegonia numerous, usually 15–18, mostly 866 μ long and 105 μ in diameter at largest portion. Spores nearly spherical, 25.5–28 μ in diameter, brownish-yellow, finely reticulate but somewhat irregularly. Elaters strict, very long, usually 155–1384 μ in length (averaging 1040 μ) and 12.7–15 μ in maximum diameter (mostly 14.8 μ), spiral only in the middle part, about one-third of the entire length, coalescing along a longitudinal line; both end-portions without any spiral, longly attenuate to slender extremities; rarely the elaters abnormally branched* or shortened. ♂ inflorescence solitary or geminate on the dorsal surface of the thallus, somewhat crescent-shaped, scales low and insignificant; antheridia aggregate, numerous, usually 20–30, nearly spherical, with a short pedicel, immersed singly in each alveolus. Spermatozoids 76–104 μ long and 2 μ in diameter, with two long cilia at the anterior portion.

Fr. April–May. Both ♀ and ♂ sexual organs are ripen in the same season.

Hab. On wet soil and decaying logs, seldom on rocks or bark, in mountainous regions. ·

---

* So far as known, the branching elaters occur in *Conocephalus, Targionia, Radula,* and *Anthoceros,* cf. TILDEN, J. E., Minnesota Bot. Stud., Bull. No. 9, pp. 43–52 (1894) & MÜLLER, K., RABENHORST's Kryptogamen-Flora, p. 231 (1907).

Loc.

Honshiu: Mt. Hakkôda, Shinyu 800 m., prov. Mutsu (Y. HORIKAWA, no. 1229, July 1928) ; Akita, prov. Ugo (U. FAURIE, 1897) ; Hiraizumi, prov. Rikuchû (Y. HORIKAWA, no. 1185 & 1186, May 1928) ; Sendai, prov. Rikuzen (Y. HORIKAWA, no. 251, Jan. & no. 268, March 1927) ; Insl. Kinkwasan, prov. Rikuzen (Y. HORIKAWA, no. 798, Aug. 1927) ; Kamo, prov. Echigo (B. HAYATA, 1893) ; Mt. Takao, prov. Musashi (K. MIYAKE, May 1899) ; Mt. Kiyozumi, prov. Awa (T. MAKINO, Apr. 1898) ; Mt. Amagisan, prov. Idsu (B. HAYATA, 1899) ; Hiroshima, prov. Aki (Y. HORIKAWA, Apr. 1924) ; Iwakuni, prov. Suwo (Y. HORIKAWA, no. 288, March 1927).

Shikoku: Prov. Tosa (T. YOSHINAGA, Apr. 1899) ; Sakawa-machi, prov. Tosa (T. YOSHINAGA, no. 1248 & 1249, July 1928).

Kiushiu: Nankwan, prov. Higo (Y. HORIKAWA, no. 62, Apr. 1923) ; Sakaki-mura, prov. Higo (Y. HORIKAWA, no. 315, Apr. 1927) ; Aida, prov. Higo (K. MAYEBARA, no. 12, Feb. 1927) ; Mt. Aoidake, prov. Hiuga (Y. HORIKAWA, no. 461, Apr. 1927) ; Yunoyama, prov. Hiuga (Y. HORIKAWA, no. 392, Apr. 1927) ; Kagoshima, prov. Satsuma (Y. HORIKAWA, no. 552, Apr. 1927).

Distrib. This genus monotypic and endemic.

Remarks. This species was originally described by STEPHANI (1897) as *Pellia crispata* basing upon a sterile specimen collected by Abbé FAURIE at Akita, a north east district of Japan. Two years later MIYAKE (1899) established the genus *Makinoa* in honour of Dr. MAKINO, who collected a fertile specimen of this species from Mt. Kiyozumi in the province Awa. Since the time of this publication, unusual attention has been paid to the peculiarities of this plant*.

The systematic position of the genus *Makinoa* is of unusual interest, because in both sexual and asexual generations this genus possesses the convergent but principal morphological characters, which are common in several other genera belonging to two families. Besides,

---

*SCHIFFNER, V., Oesterr, Bot. Zeitschr. Jahrg. 1901, Nr. 3, p. 82 (1901); EVANS, A. W., Proc. Wash. Acad. Sci., Vol. VIII, p. 141 (1906) & Bull Torrey Bot. Club, Vol. 42, p. 276 (1915); GOEBEL, K., Organographie d. Pflanzen, Erster Teil, p. 134 (1913); HAYATA, B., loc. cit. (1928).

some of the important characteristics are quite peculiar to *Makinoa*, viz. :

Sexual generation

1.	Structure of thallus	*Symphyogyna, Mörckia* (Leptotheceae)
2.	Crescent-shaped antheridial receptacle	peculiar to *Makinoa*
3.	Filamentous underleaves	*Mörckia* (Leptotheceae).
4.	Thalamogenous calyptra	*Aneura, Metzgeria* (Metzgerioideae).

Asexual generation

5.	Oblong-cylindrical capsule	*Aneura, Hymenophyton* (Metzgerioideae). *Pallavicinia, Mörckia* (Leptotheceae).
6.	Mode of capsule-dehiscence	*Pallavinia, Mörckia* (Leptotheceae).
7.	Structure of capsule-wall	*Symphyogyna* (Leptotheceae).
8.	Apical elater-bearers	*Aneura, Metzgeria* (Metzgerioideae).
9.	Structure of spores	*Pallavicinia* (Leptotheceae).
10.	Structure of elaters	peculiar to *Makinoa*.

As seen above, it will be evident that *Makinoa* has an affinity on one hand to Leptotheceae and on the other hand to Metzgerioideae. Schiffner[*] maintained the genus should be included in Leptotheceae and put it next to *Symphyogyna*. But *Makinoa* can be distinguished sharply from this genus by the following principal characters :

		Makinoa	*Symphyogyna*
1.	Antheridia	grouped in a crescent-shaped receptacle	scattered on the dorsal side of thallus.
2.	Apical elater-bearers	strongly developed	indistinct or rudimental
3.	Structure of elaters	peculiar-type	common-type
4.	Capsule-wall	2 or 3 cell-layer with annular thickenings	1 cell-layer without annular thickenings

---

[*] Schiffner, V., Oesterr. Bot. Zeitsch., Nr. 3, p. 82 (1901).

Consequently, from the phylogenetical stand-point we are able to say that our *Makinoa* has a more or less close relationship both to Metzgerioideae and to Leptotheceae, but that its systematic position will be rather distant from all the other genera, belonging to Lepto-theceae and Metzgerioideae.

Recently the observations on the age of some American Bryophytes have been reported by FRYE*. In the case of *Makinoa* the growth practically ceases in autumn, and when spring comes the plants begin to grow again. Between these two periods there is a distinct constric-tion, showing on the dorsal surface the traces of ♀ or ♂ receptacles (Pl. I, Fig. 1). This enables us to know the years of growth most easily. In this way the writer could trace at least 6 years of growth, the last 4 of which were still alive. The gametophyte is therefore an evergreen perennial.

Genus: SCHIFFNERIA STEPHANI (1894)

Schiffneria STEPHANI, Oesterr. Bot. Zeitsch., Bd. 44, p. 1 (1894).

Schiffneria viridis STEPHANI
(Pl. VIII.)

Jungermannia sp.? TSUGE, Hepat. Hakone, Idsu, Nikko, Tokyo. Mss. pp. 66–68, Pl. XXXV (1887).

Schiffneria viridis STEPHANI, Spec. Hepat., Vol. III, p. 278, (1908); GOEBEL, Organographie d. Pflanzen, Teil II, p. 603 (1915-1918).

Dioicous. Mesophyte. Plants growing in dense patches, often attaining to 21 mm. in length and 3 mm. in breadth, pale green, the 1-celled margin somewhat transparent. Stems creeping or procumbent, fleshy, arcuate, commonly simple or furcate, the branches arising from the ventral side of stem ; midrib broad, convex postically and slightly convex antically, usually 6 cells** thick in the middle, gradually passing into the 1-stratose margins, outer cells as large as the inner, averaging 85 μ in diameter. Rhizoids pellucid, simple or rarely furcate, scattered to the underside of broad stem, generally attaining to 7 mm. in length and 9–25 μ in breadth. Leaves succubous and regularly arranged,

*FRYE, T. C., Bryologist, Vol. XXXI, pp. 25-29 (1928).
**STEPHANI described originally, "Costa 8 cellulas crassa".

semi-circular in form, slightly imbricate, ventral inner margin lunately cleft. Leaf-cells plane, 5–7-angled, averaging 84 μ in diameter in the middle portion, walls thin throughout, trigones absent, the cells at margin with slightly thickened walls. ♀ inflorescence borne on a leading branch or on a lateral branch; archegonia numerous. Involucral bracts about twice as large as the leaves, canaliculate-concave, narrowly oblong, 1/4–1/3 bilobed, with 1–3 teeth on each lobe, the lobes lanceolate, acute or subobtuse; bracteoles similar to the bracts. Perianth long exserted, oblong-ovate, 5 mm. long and 1.2 mm. wide, of one layer of cells near apex, 2–3 cell-layers at middle and base, mouth somewhat constricted, irregularly lobate (usually about in 8), each lobe ciliate; calyptra obovoid, thalamogenous. Capsule oblong-cylindrical, 0.8–1.1 mm. long, 0.58–0.65 mm. broad, blackish and shiny. Splitting regularly into four equal valves. Wall of 2–3 layers of cells, inner layer with elongated cells having numerous semi-annular brownish-yellow thickenings, pedicel hyaline and slender, 19 mm. long. Spores 11–16 μ in diameter, mostly 13 μ, spherical, brownish-yellow, regularly reticulate, the lamellae low, margin of spore appearing as if crenulate-dentate, the teeth being 17–21 in number. Elaters light-red, tapering at each end, with two spiral bands, 40–300 μ long and 11–16 μ broad. Androecium terminating a short postical branch, bracts in about 2 pairs, suberect, inflated, shortly and unequally bifid with rounded divisions, closely imbricate; antheridia globose, shortly-pedicellate in each saccate base of unequally bilobed bract.

Fr. April-May.

Hab. On bark, decaying logs and stumps in moist, sheltered places in mountainous regions.

Loc.

Honshiu: Mt. Amagi, prov. Idsu (C. TSUGE, Apr. 1886); Aokigahara, the foot of Mt. Fuji, prov. Kai (B. HAYATA. 1899); Mt. Misen, Insl. Miyajima, prov. Aki (Y. HORIKAWA, no. 209, May 1924).

Shikoku: Chiyoga-tani, Onomi-mura, prov. Tosa (T. YOSHINAGA, no. 1250, Apr. 1925); Mt. Yokogura, prov. Tosa (T. YOSHINAGA, Aug. 1901); ibidem (Y. HORIKAWA, no. 176, March 1924).

Kiushiu: Mt. Aoi-dake, prov. Hiuga (Y. HORIKAWA, no. 436, Apr. 1927); Mt. Kirishima, prov. Ôsumi (Y. HORIKAWA, no. 489 & no. 490, Apr. 1927).

Distrib. This species endemic.

Remarks. The genus was established by STEPHANI in 1894, based on a single species, *Schiffneria hyalina*, which was collected by Dr. WARBURG on Mt. Sibella of Isle Batjan. It was named in honour of Prof. SCHIFFNER. Fourteen years later, STEPHANI (1908) added a second new species, *Schiffneria viridis*, based on sterile material collected by YOSHINAGA at Mt. Yokogura in the province of Tosa. Thus we have at present, two species of the genus.

Seven years before the appearance of the genus *Schiffneria* by STEPHANI, the late TSUGE (1887) already described and figured this species as *Jungermannia* sp.? basing on fertile material collected by himself on Mt. Amagi in the province of Idsu. It is to be regretted that his manuscript is not yet published.

In its morphological features the genus *Schiffneria* is one of the most remarkable and important genera of the Hepaticae. This genus represents the intermediate form just between the thallophytes and cormophytes, and for this reason, an unusual amount of attention of many other authors * has been attracted to the peculiarities of this plant.

At first STEPHANI (1894) in his paper put this genus near to *Hymenophyton*, suggesting that it represents rather the transitional form between Jungermanniaceae anacrogynae and Jungermanniaceae acrogynae. But he afterwards (1908) removed it into the Trigoniantheae, as closely allied to the genus *Odontoschisma*. So far as our present investigation goes, it is yet impossible to determine its systematic position positively. The genus appears in its morphological characteristics to have no intimate relationship to any of the other member of Trigoniantheae.

_____

* SCHIFFNER, V., Conspect. Hepat. Archip. Ind., p. 64 (1898); GOEBEL, K., Organographie d. Pflanzen, Teil 2, p. 603 (1915-1918); WETTSTEIN, R., Handb. d. syst. Bot., 2-Aufl. Bd. 1, p. 310 (1923); HAYATA, B., Bot. Magaz. Tokyo, Vol. 42, p. 181 (1828).

Genus : PLEUROZIA Dumortier (1835)

Pleurozia arcuata Horikawa, sp. nov.
(Pl. IX & Text-Fig. 2.)

Sterile.   Xerophyte.   Growing in scattered or loose depressed tufts.
Plants usually 70–87 mm. long and 2.2–2.65 mm. wide, reddish-yellow
to purple, very robust.   Stems arising from the rhizomatous base,
0.42–0.57 mm. in diameter, rather stout, flexuous, arcuate, reddish-black,
greenish-yellow near the apex, denudate at the base, closely leaved
elsewhere, suberect or decumbent, simple or sparingly branched ; in
cross-section each cell thickened in its corner, 2-3 outmost cell-layers,
cortex-cells brown, cells in the central portion much larger, colourless.

Text-Fig. 2.
Growing-habit of *Pleurozia arcuata* Horikawa, in natural size.

Rhizoids absent.   Leaves densely imbricate, concealing the stem, posti-
cally secund, incubous, complicate-bilobed.   Antical lobe very large,
2.2–3.3 mm. long and 1.7–2.2 mm. wide, patent, convex, broad-triangular,
divided up to 7/45 in two triangular segments, acute or subacute,
strongly incurved and somewhat connivent, the antical margin of lobe
broadly rounded, widely crossing the stem, decurrent, usually with
2–3 triangular spinous teeth above the base, the postical margin

slightly curved; both margins towards the apex, together with the segments, spinous-dentate. Postical lobe inflated saccate, 1.5–1.65 mm. long and 0.99–1.55 mm. wide, attached to the antical lobe in the middle with a narrow portion, the lower part incumbent on the postical side of the stem which it crosses, erect-patent and nearly parallel to the stem, ovate, narrowed at the base, hooded at the apex, the antical side turning to the stem with a longitudinal deepening, which falls into the sack by valve apparatus. Underleaves absent. Cells of antical lobes very large, at middle averaging 38 μ long and 28 μ broad, oblong, nodulose and stellate, trigones very large and sometimes confluent, reddish-yellow to purple; cells near the apex 25 μ in diameter, rounded or rounded-oblong, slightly stellate, trigones very large; cells at the base 46 μ long and 25 μ broad, linear-oblong, the trigones smaller and slender; cells at the margin 24 μ long and 20 μ broad. Cuticle nearly smooth. Remaining parts not seen.

Hab. On bark? in subalpine district.

Loc.

Formosa: Mt. Daibusan, prov. Takao (Y. SHIMADA & S. ÔHASHI, no. 15-type, Jan. 1928).

Distrib. This species endemic.

Remarks. *P. arcuata* is the first species of the genus *Pleurozia*, consequently of the family Pleurozioideae to the flora of Japan. Up to the present day eleven species of *Pleurozia* have been described from all the world.* Among these, the present species is most closely related to the widely distributed *P. purpurea* (LIGHTF.) LINDB. It agrees with *P. purpurea* in its general habit, in its strongly incurved antical lobes and in its postical inflated saccate lobes. It differs, however, from *P. purpurea* in the following points:

	P. purpurea	*P. arcuata*
1. Number of triangular spines on the postical margin of antical lobes.	1–2	2–3
2. Size of cells in antical lobes.		
at apex	18 μ	25 μ
at margin	12 × 14 μ	20 × 24 μ

*STEPHANI, F., Spec. Hepat. Vol. IV, p. 236 (1910) & Vol. VI, p. 517 (1924).

at middle	$15 \times 20\,\mu$	$28 \times 38\,\mu$
at base	$18 \times 39\,\mu$	$25 \times 46\,\mu$
trigones of cells at base	very large	smaller & slender
3. Cuticle	verruculose	smooth

Genus: FRULLANIA RADDI (1820)

Subgen. *Thyopsiella* SPRUCE (1884)

Sect. *Obtusifolioe*

The row of ocelli occurs in the antical lobe. Postical lobe cylindrical-saccate, longer than broad. Perianth smooth. Antical lobe rounded at the apex.

Key to the Japanese species

1 ⎰ Underleaves rotundate, thrice as broad as the stem, 1/10–1/7 bilobed ....*F. Makinoana*
  ⎱ Underleaves narrowly oblong, less than twice as broad as the stem, more than 1/3 bilobed 2

2 ⎰ Antical lobes decurved, ocelli short, 2–5 cells long, lobule of stem separated from it by about half its own width; lobule of branch lying obliquely, forming with the stem an angle of 45° ....*F. densiloba*
  ⎱ Antical lobes not decurved, ocelli long, more than 9 cells long, lobule of stem separated from it by about equal its own width; lobule of branch subparallel, forming with the stem an angle of less than 15° 3

3 ⎰ Plant brownish-red, ocelli 11–16 cells long, underleaves usually 1/2.6 bilobed ....*F. tsukushiensis*
  ⎱ Plant yellowish-green, ocelli 9–11 cells long, underleaves usually 1/1.7 bilobed ....*F. aoshimensis*

Frullania densiloba STEPHANI
(Text-Fig. 3.)

Frullania densiloba STEPHANI (published as a *nomen nudum* by YOSHINAGA, in Bot. Magaz. Tokyo, Vol. XV, p. 92, 1901), EVANS, Proc. Wash. Acad. Sci., Vol. VIII, p. 157 (1903); STEPHANI, Spec. Hepat., Vol. IV, p. 549 (1911).
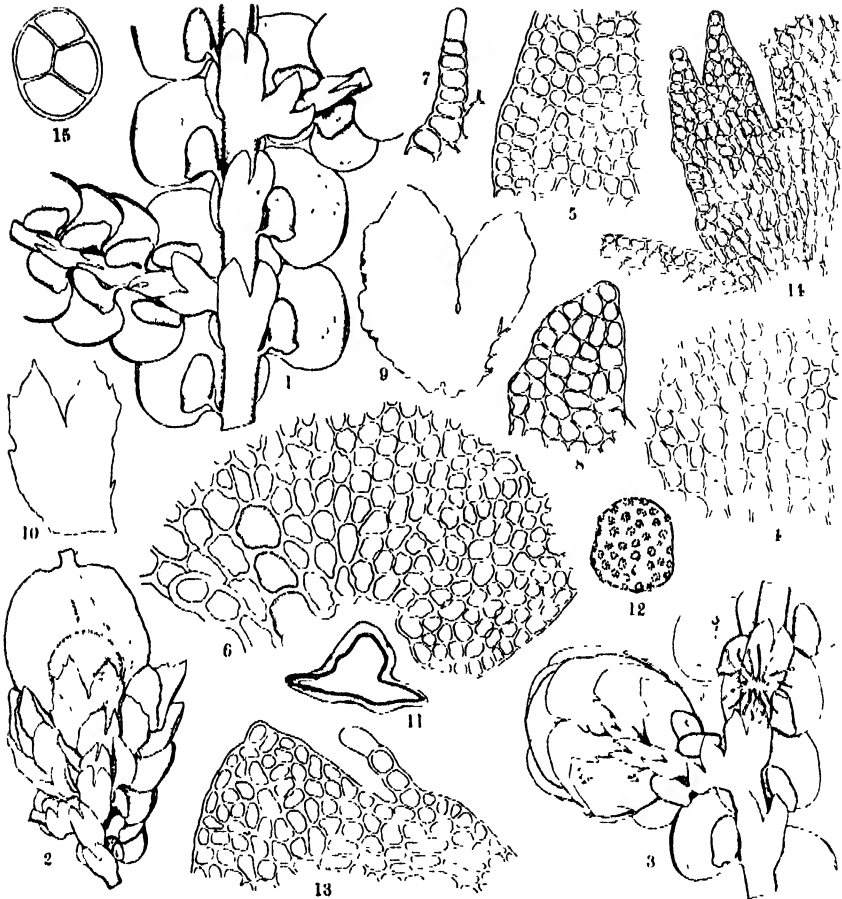
Text-Fig. 3. *Frullania densiloba* STEPHANI

1, Part of plant with bases of 2 branches, postical view, ×36. 2, Part of ♀ plant with perianth, postical view, ×23. 3, Part of ♂ plant with androecium, postical view, ×36. 4, Cells from middle of antical lobe, ×233. 5, Cells from margin of antical lobe, ×233. 6, Base of antical lobe, ×233. 7, Stylus of stem-leaf, ×233. 8, Apex of one division from a stem-underleaf, ×233. 9, Innermost bract of ♀ inflorescence, ×38. 10, Innermost bracteole of ♀ inflorescence, ×38. 11, Cross-section of perianth in upper third, ×23. 12, Spore, ×300. 13, Postical lobe of bract of androecium with 3-celled tooth, ×233. 14, Innermost bracteole of androecium showing the state of being connated with the bract on one side at the base, ×144. 15, Gemma, ×233.

Dioicous. Xerophyte. Plants small, usually 30 -35 mm. long, dark red to yellowish-brown, glossy, growing in depressed mats. Stems fragile and flexuous, prostrate, rather firmly adherent to the substratum, about 118 μ in diameter, reddish-brown, at first regularly pinnate with short, obliquely to widely spreading branches, some of the branches remaining short and simple, others becoming themselves pinnate in the same way as the stem. Rhizoids fasciculate, colourless, rather scarce. Stem-leaves contiguous to loosely imbricated, the lobes widely spreading, somewhat falcate, oblong-obovate, averaging 388 μ long and 266 μ wide, slightly convex, rounded at the antical base and arching far across the stem, the apex rounded and decurved, margin entire. Lobule clavate, about 165 μ long and 105 μ wide, inflated throughout, subparallel with the stem and separated from it by about half its own width, mouth obliquely rounded. Stylus minute, filiform, or subulate, tipped with a hyaline papilla, mostly 4 or 5 cells long and 1 or 2 cells wide at the base. Branch-leaves smaller than the stem-leaves, averaging 319 μ long and 238 μ wide, relatively more closely imbricated, lobules similar to those of the stem but closed together and more oblique, lying with their rounded ends upon the axis and forming with it an angle of about 30°. Leaf-cells roundish, plane or nearly so, averaging about 10.5 μ at the margin of the lobe, 14.5 μ in the middle and 17 × 30 μ at the base, walls somewhat thickened, trigones small, the portion lining the cavity being usually pigmented. Cuticle smooth. Ocelli mostly in a single row of 2–6 cells, running obliquely from the stem between the axis of the lobe and the postical margin, averaging 23 × 28 μ in size, contents dark red, ocelli of leaves subtending branches often in 2 rows. Underleaves of the stem distant, narrowly oblong, averaging 250 μ long and 166 μ wide, neither cordate nor rounded at the base, bifid nearly one-half or less with a narrow acute sinus and broad, erect, rounded divisions, the margins always plane, entire. Underleaves of the branches contiguous to subimbricated, often partially covered over by the lobules, narrowly ovate or ligulate, 185 μ long and 93 μ wide, with narrow and often acute divisions. ♀ inflorescence borne on a leading branch. Involucral bracts 2–4 pairs, passing by insensible gradations into the leaves, complicate and unequally bifid. Lobes of the innermost bracts ovate to oblong, 736.66 μ long and 394 μ wide, narrowed toward the apex but usually obtusely

pointed, margin irregularly sinuate. Ocelli not existing. Lobule of innermost bracts ovate-lanceolate, 591 μ long and 275.7 μ wide, acute or subacute at the apex, margin irregularly sinuate, usually bearing a cluster of short and irregular cilia above the base. Innermost bracteole free, ovate, averaging 570 μ long and 420 μ wide, bifid to nearly the middle with a narrow sinus and acute divisions, margin distinctly short-ciliate. Perianth reddish-brown, 1.1 mm. long and 0.75 mm. wide, about 2/3-exserted, obovate or pear-shaped in outline, trigonous, nearly smooth, gradually narrowed toward the base, rounded to truncate at the apex, beak short, cylindrical, entire or nearly so at the mouth, perianth compressed, but with a distinct, rounded postical keel, narrow toward the apex, surface nearly smooth. Capsule globose, pedicel very short. Spores averaging 38.28 μ in diameter, roundish or oblong, usually somewhat obtusely angular, pale brown, with several large tubercular warts. Elaters reddish-brown. Male plant separate ; Androecium on a short lateral branch, globose, averaging about 186 μ in diameter, bracts 3-4 pairs, imbricate, ventricose, antical lobe broadly ovate-oval, rounded at the apex ; postical lobe rather smaller, ovate somewhat falcate, rounded-obtuse, with a usually 3-celled tooth near the apex, the tooth tipped with a hyaline papilla ; bracteole 2–3, lanceolate, 262 μ long and 92 μ wide, 1/5 bilobed, connate with the bract on one side at the base. Gemmae rare, on the margin and surface of the antical lobe, roundish, multi-cellular.

Fr. April-May.

Hab. On rocks or bark of trees in mountainous regions.

Loc.

Honshiu : Insl. Miyajima, prov. Aki (Y. HORIKAWA, no. 211, May 1924 & no. 704, no. 710, Apr. 1927) ibidem (A. NOGUCHI, no. 102, Nov. 1927).

Shikoku : Mt. Ishiduchi, prov. Iyo (S. OKAMURA, no. 119, Aug. 1904) ; Mt. Yokogura prov. Tosa (T. YOSHINAGA, no. 23, Aug. 1901) ; Mt. Kônomine, prov. Tosa (T. YOSHINAGA, no. 32-type, Nov. 1903) ; Sada-misaki, prov. Tosa (T. YOSHINAGA, Aug. 1899).

Kiushiu : Mt. Aoidake, prov. Hiuga (A. NOGUCHI, no, 77, Jan. 1928) ; Mt. Kirishima, prov. Ôsumi (Y. HORIKAWA, no. 527, Apr. 1927).

Distrib. The species endemic.

Frullania aoshimensis Horikawa, sp. nov.
(Text-Fig. 4)

Sterile. Xerophyte. Plants small usually 15 mm. long, green, glossy, growing in rather depressed mats. Stems fragile and prostrate, rather firmly adherent to the substratum, about 98.5 μ in diameter, green, at first regularly pinnate with short, obliquely to widely spreading branches, some of the branches remaining short and simple, others becoming themselves pinnate in the same way as the stem. Rhizoids fasciculate, colourless, rather numerous. Stem-leaves imbricate, the lobes widely spreading, somewhat falcate, oblong-obovate, averaging 472 μ long and 426 μ wide, somewhat convex, rounded at the antical and arching rather far across the stem, the apex rounded and not decurved, margin entire. Lobule clavate, 160 μ long and 92 μ wide, inflated throughout, parallel or subparallel with the stem and separated from it by nearly equal or less (averaging 0.8) its own width, mouth obliquely rounded. Stylus, minute, filiform or subulate, tipped with a hyaline papilla, mostly 4–6 cells long and 1 or 2 cells wide at the base. Branch-leaves smaller than the stem-leaves, averaging 344 μ long and 263 μ broad, more closely imbricated, with a spine at the postical margin of the branch-axil-leaves, usually three cells long and 2 cells wide at the base, lobules similar to those of the stem but more closed together and more or less subparallel, lying with their rounded ends upon the axis and forming with it an angle of about 22'. Leaf-cells containing numerous chloroplasts, plane or nearly so, averaging about 10 μ at the margin of the lobe, 13 μ in the middle and 9 × 28 μ at the base, walls more or less thickened and with distinct but small trigones and intermediate thickenings except toward the base. Ocelli mostly in a single row of 9–12 cells, running obliquely from the stem between the axis of the lobe and the postical margin, averaging 22.5 × 30 μ in size, contents dark red. Cuticle smooth. Underleaves of the stem distant, narrowly oblong, averaging 243 μ long and 184 μ wide, neither cordate nor rounded at the base, 1/1.77 bilobed, the sinus subacute or obtuse, divisions erect, acute or subacute apex, the margins plane and entire. Underleaves of the branches contiguous to subimbricated, never covered over by the lobules, narrowly ovate or igulate, 170 μ long and 85 μ wide with narrow and acute divisions.
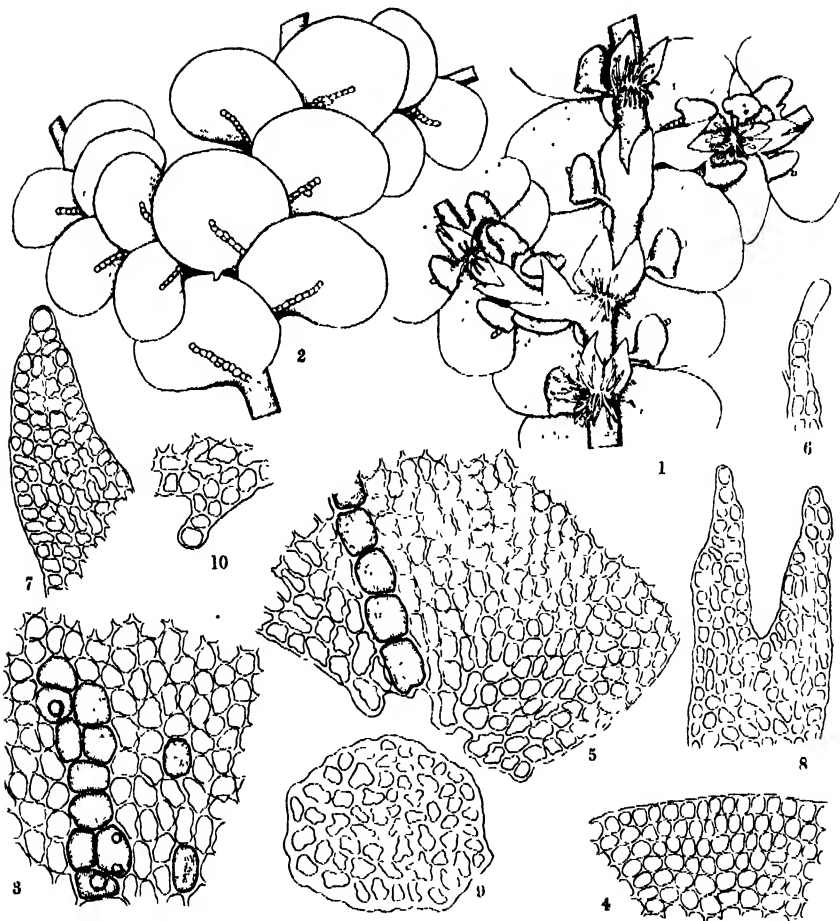
Text-Fig. 4. *Frullania aoshimensis* HORIKAWA

1, Part of plant with bases of 2 branches, postical view, ×38. 2, Part of plant with 2 bases of branches, antical view, ×38. 3, Cells from middle of antical lobe, including ocelli, ×233. 4, Cells from margin of antical lobe, ×233. 5, Base of antical lobe, ×233. 6, Stylus of stem-leaf, ×233. 7, Apex of one division from a stem-underleaf, ×233. 8, Branch-underleaf, ×233. 9, Cross-section of stem, ×233. 10, Spine of postical margin of antical lobe, ×233.

Hab. On bark of *Ardisia Sieboldi* MIQ. (Moku-tachibana) at the seashore.

Loc.

Kiushiu: Insl. Aoshima, prov. Hiuga (Y. HORIKAWA, no. 357-type, Apr. 1927).

Distrib. The species endemic.

Frullania tsukushiensis HORIKAWA, sp. nov.
(Text-Fig. 5.)

Dioicous. Xerophyte. Plants small, usually 15 mm. long and 0.955 mm. wide, brownish-red, glossy, growing in depressed mats. Stems fragile and flexuous, prostrate, firmly adherent to the substratum, about 100 μ in diameter, reddish-brown, densely branched, at first regularly pinnate with short, obliquely spreading branches, most of the branches remaining short and simple, others becoming themselves pinnate in the same way as the stem. Rhizoids fasciculate, colourless, rather dense. Stem-leaves rather closely imbricated, the lobes widely spreading, somewhat falcate, ovate (obovate), averaging 512 μ long and 234 μ wide, somewhat convex or nearly plane, rounded at the antical base and slightly across the stem, the apex rounded and not decurved, margin entire. Lobule clavate, about 170 μ long and 92 μ wide, inflated throughout, parallel with the stem and separated from it by about equal its own width, the mouth obliquely rounded. Stylus minute, filiform, tipped with a hyaline papilla, mostly 3 or 5 cells long and 1 or 2 cells wide at the base. Branch-leaves smaller than the stem-leaves, averaging 420 μ long and 282 μ wide, more closely imbricated, lobules similar to those of the stem but closed together and more or less subparallel, lying with their rounded ends upon the axis and forming with it an angle of about 15°. Leaf-cells plane or nearly so, averaging 9 μ at the margin of the lobe, 15.3 μ in the middle and 12 × 31 μ at the base, walls more or less thickened and with distinct small trigones and intermediate thickenings except toward the base. Cuticle nearly smooth. Ocelli mostly in a single or two rows of 11–16 cells, rarely in three rows, running obliquely from the stem between the axis of the lobe and the postical margin, averaging 17.6 μ in diameter, contents dark red. Underleaves of stem subdistant or loosely imbricated, narrowly oblong, averaging 292 μ long and 21.4 μ wide, neither cordate nor rounded at the base, 1/2.6 bilobed, the sinus acute, divisions erect, acute or subacute apex, the margins plane and nearly entire. Under-

leaves of the branches imbricated, narrowly ovate or ligulate, averaging 232 μ long and 120 μ wide, with narrow and acute divisions. ♀
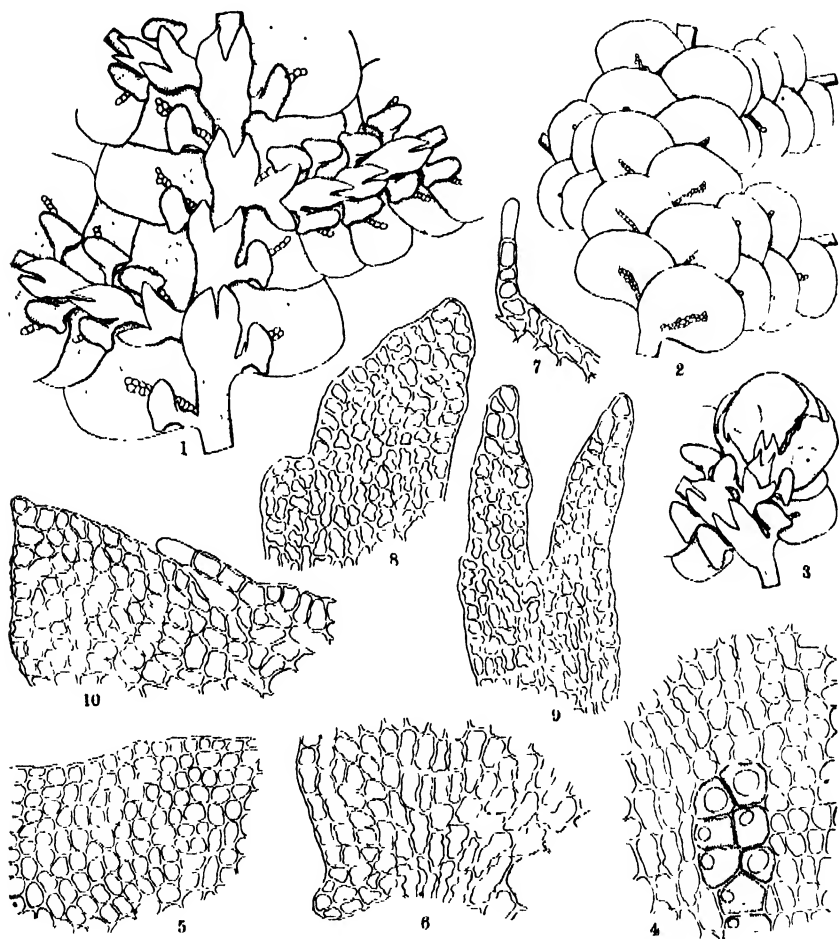
Text-Fig. 5. *Frullania tsukushiensis* HORIKAWA

1, Part of plant with bases of 3 branches, postical view, ×38. 2, Part of plant with bases of 3 branches, antical view, ×23. 3, Part of ♂ plant with androecium, postical view, ×38. 4, Cells from middle of antical lobe, including ocelli, ×233. 5, Cells from margin of antical lobe, ×233. 6, Base of antical lobe, ×233. 7, Stylus of stem-leaf, ×233. 8, Apex of one division from a stem-underleaf, ×233. 9, Branch-underleaf, ×233. 10, Postical lobe of bract of androecium with 3-celled tooth, ×233.

inflorescence not seen. Androecium on a short lateral branch, brownish-red, globose, averaging about $525\,\mu$ in diameter, bracts 2–3 pairs, imbricate, ventricose, antical lobe broadly ovate-oval, rounded at the apex; postical lobe rather smaller, ovate, apex obtuse with a mammi-form papilla, bearing a usually 3–4-celled tooth near the apex, the tooth tipped with a hyaline papilla; bracteole 2, lanceolate, $275\,\mu$ long and $120\,\mu$ wide, 1/3 bilobed, connate with the bract on one side at the base. Antheridia globose, $145\,\mu$ in diameter.

Hab.   On bark in mountainous region.

Loc.

Kiushiu: Mt. Aoidake, prov. Hiuga (Y. HORIKAWA, no. 452-type & no. 532-cotyc, Apr. 1927).

Distrib.   This species endemic.

Frullania Makinoana STEPHANI
(Text-Fig. 6.)

Frullania Makinoana STEPHANI, Bull. Herb. Boissier, p. 89, (1897).

Dioicous.   Xerophyte.   Plants small, usually 30 mm. long, brownish-red, glossy, growing in loosely depressed mats.   Stems somewhat rigid and prostrate, closely attached to the substratum, about $130\,\mu$ in diameter, reddish-brown, usually bipinnate.   Rhizoids fasciculate, colourless, rather numerous. Stem-leaves imbricate, the lobes widely spreading, somewhat falcate, obovate, averaging $787\,\mu$ long and $736\,\mu$ wide, slightly convex, rounded at the antical base and arching far across the stem, the apex rounded and decurved, margin entire.   Lobule clavate, $230\,\mu$ long and $133\,\mu$ wide, inflated throughout, subparallel with the stem and separated from it by about equal its own width, mouth obliquely rounded.   Stylus filiform, mostly 4 cells long and of a single row of cells, tipped with a hyaline papilla and semicircular appendage.   Branch-leaves smaller than the stem-leaves, averaging $550\,\mu$ long and $433\,\mu$ wide, imbricated in the same degree as in the stem, lobules similar to those of the stem.   Leaf-cells plane or nearly so, averaging about $12\,\mu$ at the margin of the lobe, $16\,\mu$ in the middle and $16 \times 32\,\mu$ at the base, walls more or less thickened and with distinct but small trigones and intermediate thickenings except toward the base.   Ocelli mostly in 1 or 2 rows, sometimes 3 or 4 rows,
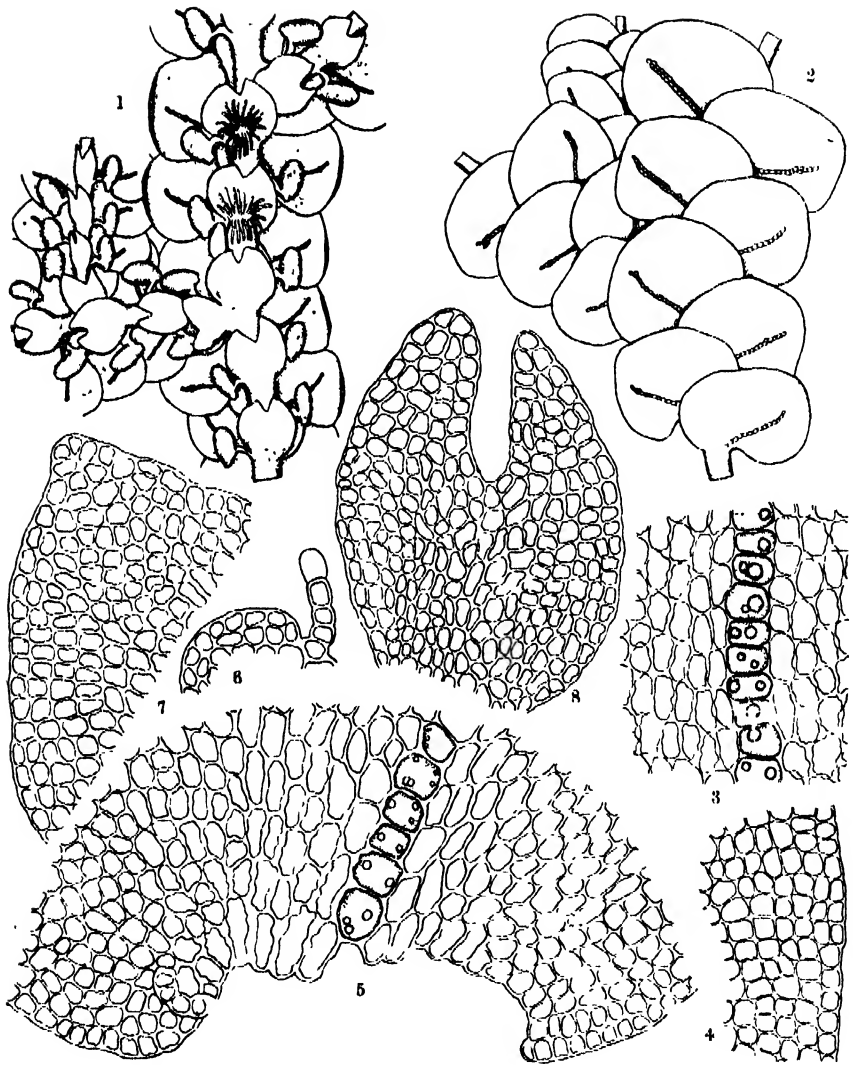
Text-Fig. 6. *Frullania Makinoana* STEPHANI

1, Part of plant with bases of 2 branches, postical view, ×23. 2, Part of
plant with base of branch, antical view, ×23. 3, Cells from middle of antical
lobe, including ocelli, ×233. 4, Cells from margin of antical lobe, ×233. 5,
Base of antical lobe, ×233. 6, Stylus of stem-leaf, ×233. 7, Apex of one
division from a stem-underleaf, ×233. 8, Branch-underleaf, ×233.

mostly 18–25 cells in length, running obliquely from the stem between
the axis of the lobe and postical margin, averaging $22.5\,\mu$ in diameter
in size, contents dark red. Cuticle smooth. Underleaves of the stem
distant, large, about thrice as broad as the stem, rotundate, averaging
$420\,\mu$ long and $394\,\mu$ wide, shortly decurrent, 1/10 to 1/7 bilobed, the
sinus subacute, lobes broad, erect and subobtuse, margin always plane
and entire. Underleaves of the branches similar to those of the stem-
leaves, but smaller than the stem-underleaves, $290\,\mu$ long, and $269\,\mu$
wide.

Hab. On rocks in mountainous regions.

Loc.

Honshiu: Insl. Kinkwasan, prov. Rikuzen (Y. HORIKAWA, no. 839,
Aug. 1927) ; Awa, Naukai (T. MAKINO, without definite place and
date)

Distrib. The species endemic.

EXPLANATION OF PLATE VI.

Makinoa crispata (STEPH.) MIYAKE

Fig. 1. Habit of ♀ plant bearing sporophytes, in natural size.
Fig. 2. ♂ plant with antheridial inflorescence, in natural size.
Fig. 3. Cross-section of thallus, ×8.
Fig. 4. Middle portion of thallus, in cross-section, ×75.
Fig. 5. Underleaves, ×50.
Fig. 6. Longitudinal sagittal section of ♀ inflorescence, ×23.
Fig. 7. Longitudinal sagittal section of ♂ inflorescence, ×23.
Fig. 8. Involucre, surface view, ×16.
Fig. 9. Cross-section of calyptra, upper part, ×50.
Fig. 10. Dehiscent capsule, showing valve being bended backwards and apical elaters stand up straightly, in natural size.
Fig. 11. Spermatozoids, ×740.

EXPLANATION OF PLATE VII.

Makinoa crispata (STEPH.) MIYAKE

Fig. 12. Capsule, ×8.
Fig. 13. Longitudinal section of mature capsule, showing apical elater-bearers, ×12.
Fig. 14. Elater-bearers, ×144.
Fig. 15. Cross-section of capsule-wall, ×233.
Fig. 16. Longitudinal-section of the same, ×233.
Fig. 17. Spore, surface view, ×740.
Fig. 18. Normal elater, ×300.
Figs. 19, 20. 21 and 22. Abnormal elaters. ×300.

EXPLANATION OF PLATE VIII.

Schiffneria viridis STEPHANI

Fig. 1. Habit of fertile plant, in natural size.
Figs. 2, 3. Portion of plant bearing sporophyte, antical view, ×8.
Fig. 4. Ditto, postical view, ×8.
Fig. 5. Thallus with young shoot, postical view, ×8.
Fig. 6. Cross-section of thallus, ×38.
Fig. 7. Leaves, postical view, ×38.
Fig. 8. Apex of leaf, ×144.
Fig. 9. Cross-section of perianth, near the middle part, ×38.
Fig. 10. A lobe of perianth, ×50.
Fig. 11. Part of plant with antheridial spike, postical view, ×5.
Fig. 12. Antheridial spike, antical view, ×21.

Fig. 13. Antheridium, in optical section, ×60.
Fig. 14. Spore, outer face, ×1000.
Fig. 15. Elater, ×144.

EXPLANATION OF PLATE IX.

Pleurozia arcuata HORIKAWA

Fig. 1. Part of plant near apex, antical view, ×8.
Fig. 2. Ditto, postical view, ×8.
Fig. 3. Outlines of cross-section of two stem, ×8.
Fig. 4. A portion of ditto, ×144.
Fig. 5. Antical lobe, dissected from the stem, ×8.
Fig. 6. Postical lobe, separated from the antical lobe, ×23.
Fig. 7. Ditto, side view, ×23.
Fig. 8. Cells from middle of antical lobe, ×233.
Fig. 9. Apex of an antical lobe, ×233.
Fig. 10. Uppermost marginal-spine of antical lobe, ×233.
Fig. 11. Lowermost maginal-spine of the same lobe, ×233.
Fig. 12. Outer valve, ×144.
Fig. 13. Inner valve, ×144.
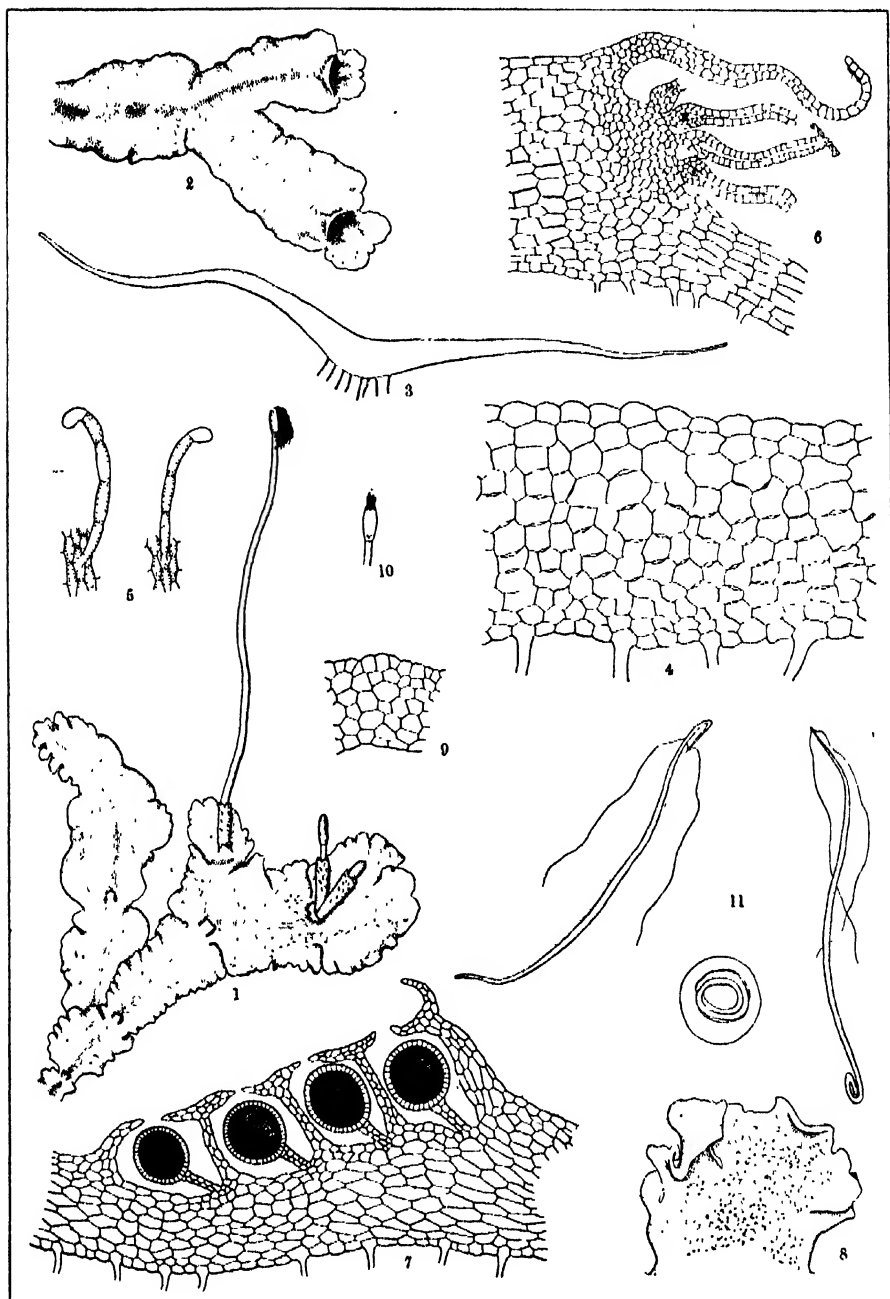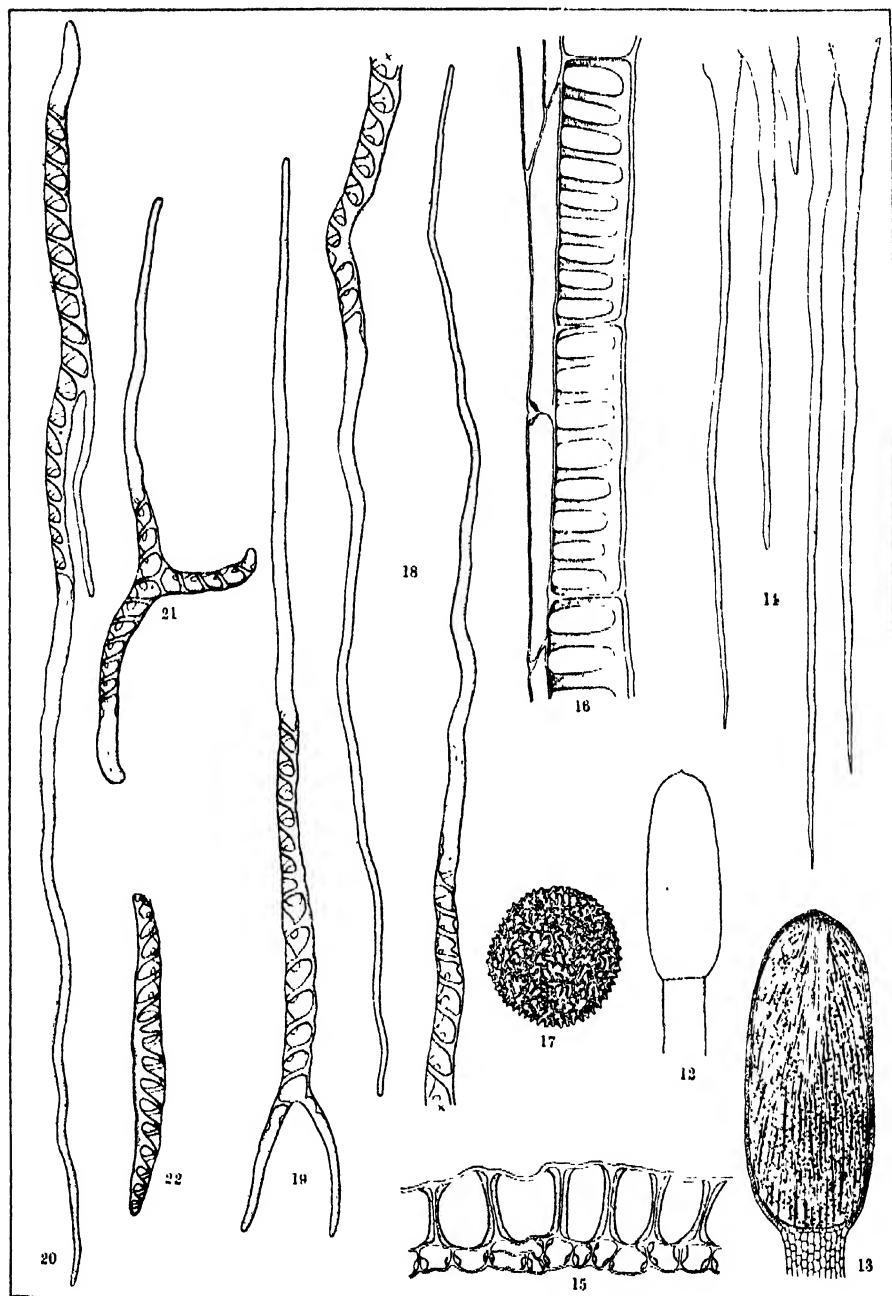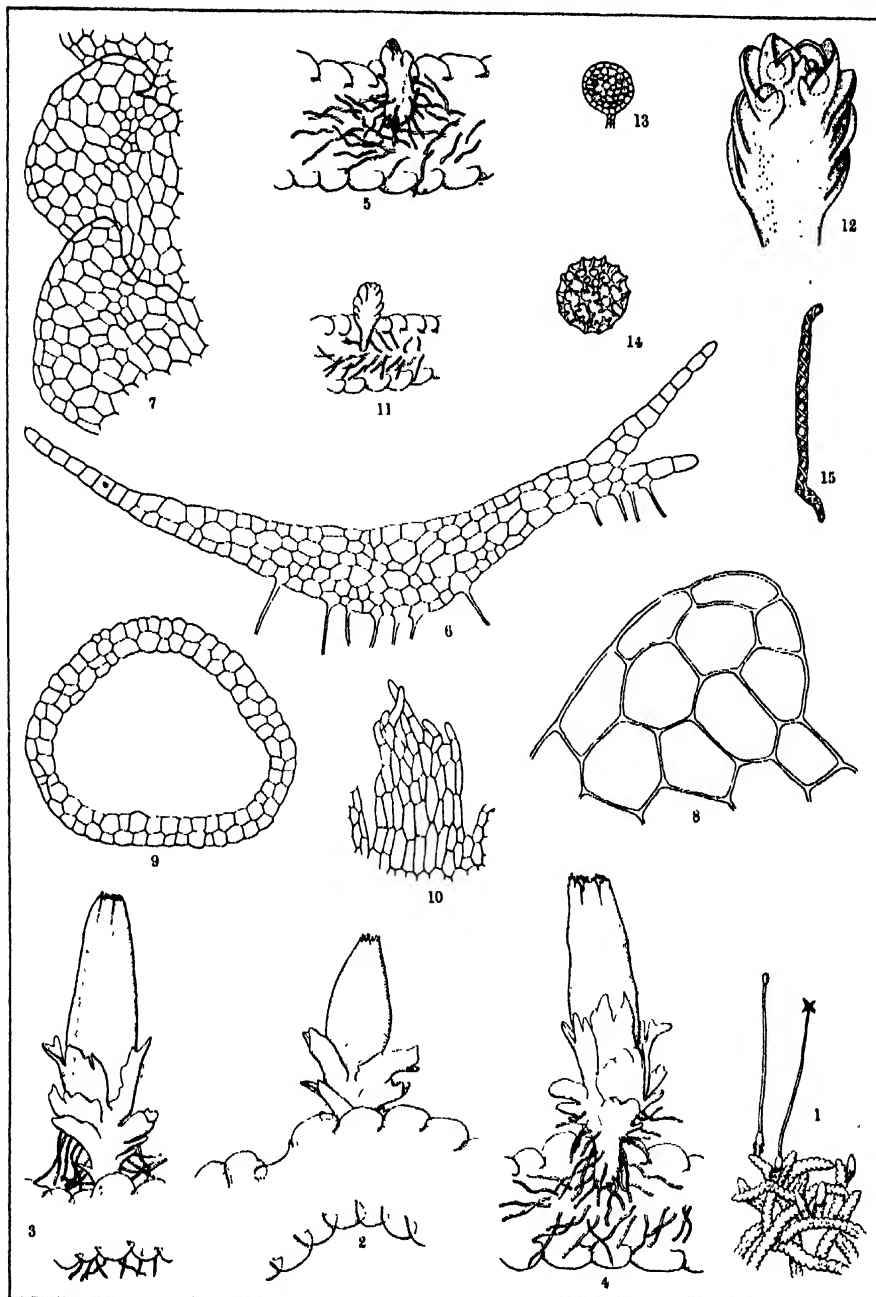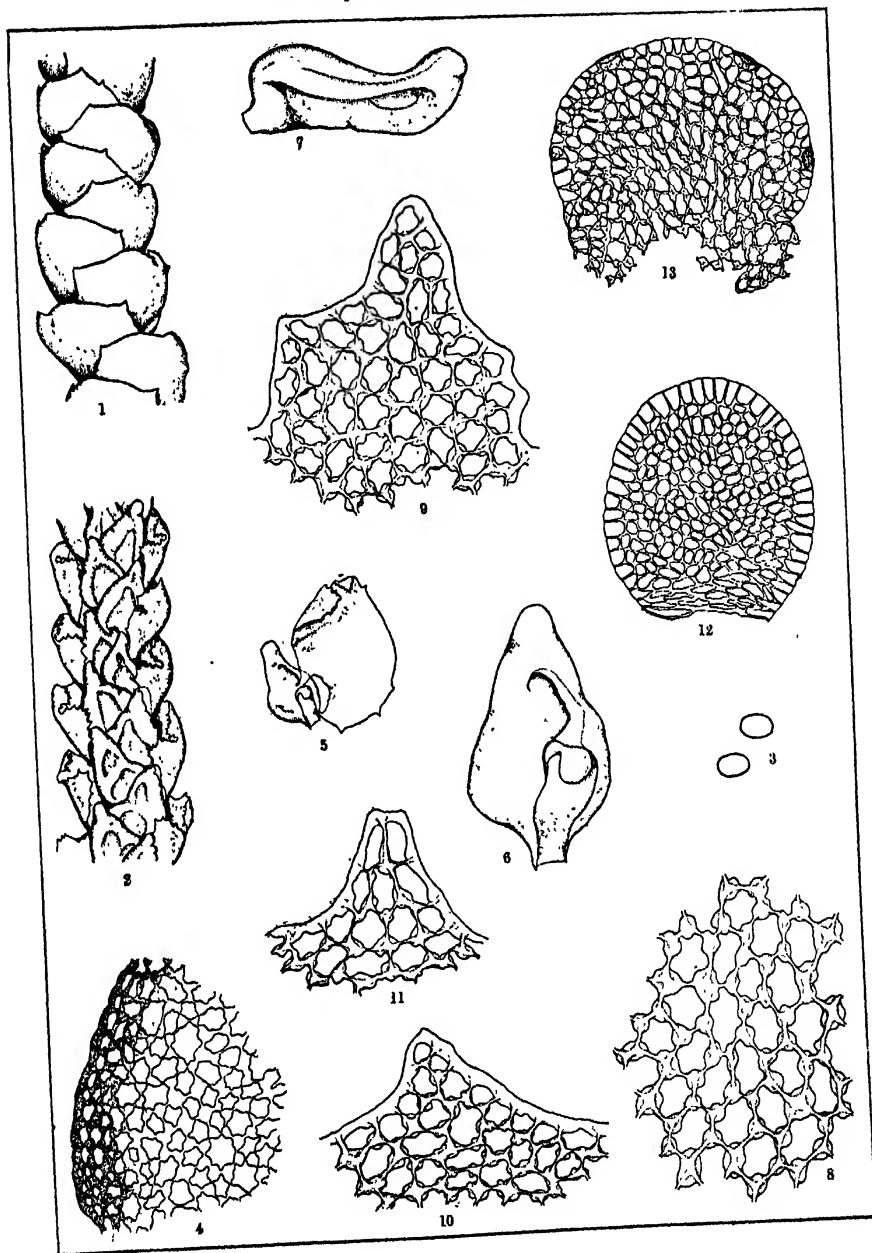
Author del.

Author del.

Y. HORIKAWA: *Pleurozia arcuata* sp. nov.

Notes on the Behavior of the Holothurian, *Caudina chilensis* (J. MÜLLER).[1]

BY

TOSHIHIKO YAMANOUCHI.

(Zoological Laboratory, College of Science, Kyoto Imperial University).

Following my study of this holothuria during the summer of 1925, I carried out these observations and experiments during the whole summers of 1926 and 1927 at the Asamushi Marine Biological Station. The contents of this report are arranged as follows : —

---

[1] A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

I. ACTIVITY.

1. EGESTION OF SAND.

The whole body of *Caudina*, as previously reported (YAMANOUCHI 1926 p. 86 Fig 1.), is buried in the sand except for the tip end of the tail, which is directed upward, just on the surface of the sea bottom. We can easily see in the sea water this end of the tail or cloaca, making pulsations for respiration. At intervals the expiration occurs with a very great amplitude, and the holothuria spouts, together with expiratory water, a stream of the egested sand to the height of a few centimetres. I made field observations to determine accurately the intervals of this spouting (Table 1).

TABLE   1.

Successive intervals of the egestions of sand in the natural habitat.
Time in minutes and seconds. Measured on Sept. 2. 1926.
10-11,30 A. M. Temp. 23°C. 5 animals.

A	B	C	D	E
4, 13	3, 17	3, 58	3, 30	4, 48
3, 10	3, 31	4, 39	3, 25	4, 56
3, 34	2, 53	4, 31	8, 30	4, 15
4, 21	3, 21	3, 17	8, 12	10, 19
4, 36	5, 07	2, 56	2, 07	5, 07
3, 43	3, 26		10, 15	4, 00
	4, 01			
	3, 19			
	2, 39			
	3, 30			
	2, 21			
	3, 14			

Sept. 2.   3-5 P. M. Temp. 24.5°C. 6 animals.

F	G	H	K	L	M
6, 53	3, 00	3, 37	3, 45	2, 19	3, 58 ·
4, 24	3, 09	3, 11	4, 04	4, 33	6, 40
5, 29	4, 26	3, 27	2, 51		3, 31
4, 56	3, 04	2, 10	4, 49		4, 36
9, 06	5, 29	5, 16	4, 38		5, 31
			3, 46		
			4, 30		
			4, 11		
			3, 54		
			3, 54		

August 30. 1–2 P. M. Temp. 23.5°C. 10 animals.

1	2	3	4	5	6	7	8	9	10
1,38	1,45	2,05	2,30	2,42	3,01	3,05	3,53	4,15	6,30

Table 1 indicates that the intervals vary greatly with different individuals, and even with a single individual. These variations, however, are within certain limits; the most of the above 77 intervals obtained from 21 animals range between 2 and 5 minutes. The most frequent interval is 3.5 minutes (Table 2).

TABLE 2.

Interval in minutes	Number of Cases
1– 2	2
2– 3	12
3– 4	29
4– 5	20
5– 6	6
6– 7	3
7– 8	0
8– 9	2
9–10	1
10–11	2
Total	77
Number of animals	21

The egestion of the sand is performed day and night. The time from the ingestion to the egestion was determined both directly by measurement and indirectly by calculation.

Direct measurement. A normal individual was placed in a large clean dish and after a time (one hour) the egested sand was gathered and weighed (I must thank my friend Dr. TAO for suggesting this method). The sum of the egested sand and the remaining contents of the intestine gives the total content of the sand. Dividing the latter by the egested sand, the time of the egestion of the total sand was directly determined.

Indirect measurement. In the previous paper (1. c. p. 91), it was noted that the animal egestes 6.57 grams of sand (weighed in the

TABLE 3.

Time in which the content of the alimentary canal is totally egested.
Aug. 1–2. 1927.	24–25°C.

Body weight without sand, in grams	Sand in the alimentary canal, in grams	Sand egested within one hour, in grams	Hours total sand egested
32.59	19.85	7.79	2.55
27.63	21.59	8.45	2.49
19.13	11.92	4.48	2.66
about 22	16.85	5.98	2.82
22.82	15.59	4.06	3.84
Mean			2.87

dry state) per hour. 6.57 grams of dry sand weighed 8.04 grams in the wet state (containing 22.3% water). The mean content of the sand measured in 170 animals was 15.16 grams; dividing 15.16 grams by 8.04 grams, we have the mean time of the egestion of the total sand content in the alimentary canal,

$$\frac{15.16}{8.04} = 1.89 \ (hours).$$

Comparing the two results,

directly measured	2.87 hours
calculated	1.89 hours

it must be pointed out that in the experimental condition the egestion of the sand proceeds more slowly than in the natural state.

2. BURROWING INTO SAND.

In any case when this animal is dragged out and placed on the sand in sea water, the anterior end of the trunk shows strong positive geotropism, and the animal returns gradually into the sand by a contracting wave of the body musculatures. The burrowing time was only recorded for the trunk, for the time of burial of the tail showed irregularity, and also the determination of the end point was difficult. The measurement was made in sea water in the afternoons of five calm days. Four or six animals (according to size) were placed on sand in each rectangular case made of tin-plate. Three such cases were placed side by side in sea water.

TABLE 4.

Mean time in minutes from the beginning of the positive geotropism
to the complete burial of the trunk in sand. Temp. 23–27°C.

Body weight without sand, in grams	Number of animals	Trunk length calculated*, in cms.	Successive trials					
			I	II	III	IV	V	VI
0– 2.5	7	2.8	6.6	7.3	7.6	8.5	9.9	9.9
2.5– 5.0	12	3.5	9.5	11.4	12.7	13.2	15.2	17.0
5.0–10.0	12	4.5	9.1	10.8	14.1	16.0	18.3	20.8
10.0–20.0	9	6.1	11.2	13.7	15.8	16.5	21.0	22.9
20.0–30.0	8	7.2	12.4	17.4	21.8	25.3		
30.0–40.0	5	8.1	17.0	21.0	26.0	33.0		
40.0–50.0	4	8.8	17.3	24.0	31.5	41.0		

*Calculated from the body weight by $Ltr = (2.38 \mp 0.10)W^{\frac{1}{3}}$ (cited from YAMANOUCHI 1929).
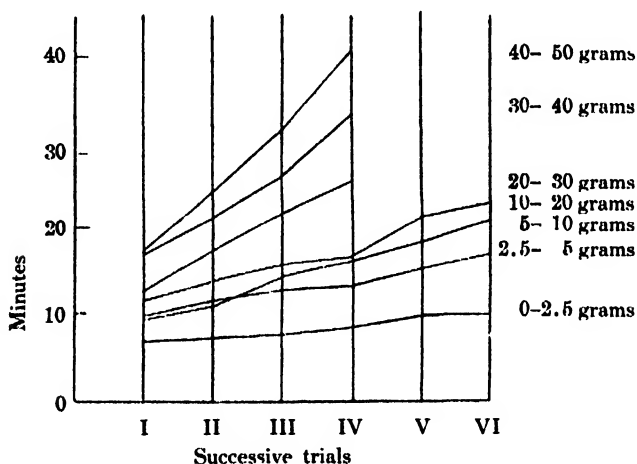
Fig. 1. Drawn from the Table 4.

The heavier or the older animals burrow more slowly than the lighter or younger ones, and by successive trials the burrowing time increases gradually. The time of the burrowing of the unit length of the trunk is not very different in any of the animals. By successive trials the velocity decreases gradually. In the animals above 20 grams this decrease is marked (Table 5).

Special attention must be paid to the high activity of the animals of 10–20 grams (without sand); in these animals the burrowing velocity

TABLE 5.

Time in minutes, in which one cm. of the trunk of the
animal burrows into sand.

Body weight without sand, in grams	Trunk length calculated, in cms.	Burrowing time Trunk length					
		I	II	III	IV	V	VI
0- 2.5	2.8	2.3	2.6	2.7	3.0	3.5	3.5
2.5- 5.0	3.5	2.7	3.3	3.6	3.8	4.3	4.8
5.0-10.0	4.5	2.0	2.4	3.1	3.6	4.1	4.6
10.0-20.0	6.1	1.8	2.2	2.6	2.7	3.4	3.7
20.0-30.0	7.2	1.7	2.4	3.0	3.5		
30.0-40.0	8.1	2.1	2.6	3.2	4.1		
40.0-50.0	8.8	2.0	2.7	3.6	4.7		
Mean		2.09	2.60	3.11	3.63	3.82	4.15

is the greatest in many cases of successive trials. The measurement
of reaction time for chemical stimuli revealed the same tendency
(Table 24). Statistical studies also revealed interesting peculiarities
among these animals (YAMANOUCHI 1929).

Burrowing into the sand is accomplished by the cooperative activity
of both the musculatures and the tentacles, for the animals whose
tentacles were cut off could no longer burrow into the sand, even
though the anterior part of the trunk showed definite positive geotro-
pism. GEROULD (1896) describes that *Caudina arenata* can completely
burrow into sand after the elapse of two or three hours; but his
observation was probably made on very tired animals.

3. LOCOMOTION.

It never occurs that the animal comes out of the sand on its own
accord, or crawls on the sand or other flat surfaces. I observed,
however, a queer crawling movement in the evening of August 7th
1926, when the animal was brought out of water and placed on a
glass plate in the air. The mode of movement was similar to its
burrowing movement into sand. The direction of the locomotion was
postero-anterior. A brief sketch of the locomotion is given in the
following text figure. The mean interval of the successive waves was
33 seconds. The interesting point is that complete retraction of the

tentacles occurs at the end of the wave. This complete retraction seems to correspond to taking the sand into the mouth. The tentacles are the most important organ for movement in the sand, and swallowing the sand by the aid of tentacles would much facilitate its movement in addition to its food taking.
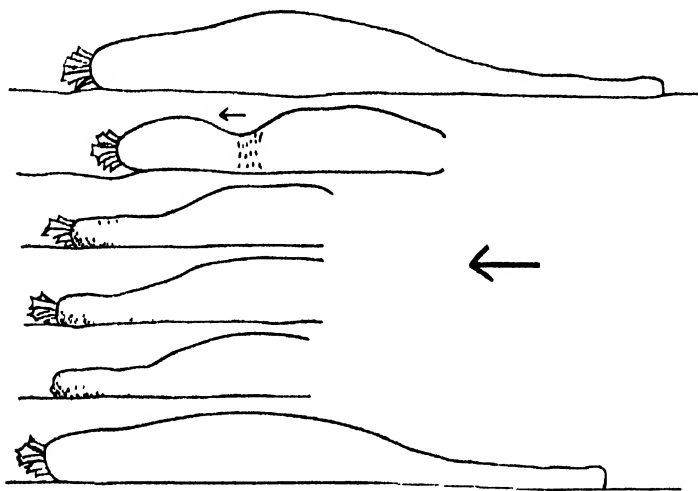
Fig. 2. Locomotion on a glass plate. Direction of the movement is indicated by the arrow.

Locomotion of the holothuria is discussed in detail by CROZIER (1915 b). No fundamental difference was observed in the case of *Caudina* from CROZIER's description. I observed in many cases when the animal was placed in water, that the peristaltic waves proceeded postero-anteriorly; such waves were also newly generated from the spot strongly stimulated on the middle trunk, as by a strong mechanical stimulus or by an acid or salt solution. Occasionally the waves proceeded in an antero-posterior direction from the stimulated spot. Posterior transmission of the stimulus is described by CROZIER in *Holothuria surinamensis* and confirmed by his studies on the rhythmic pulsation of the cloaca of *Stichopus moebii*, in which the impulse is transmitted alternately both posteriorly and anteriorly in a single pulsation (CROZIER 1916 a, p. 325).

II. MECHANICAL STIMULI.

The material was collected just before conducting the experiments and was placed on a cloth spread over the edge of a glass jar of about 2 litre content. Only the portion to be stimulated was exposed to the air, if necessary, by the adjustment of the cloth, and after the stimulation it was immediately lowered into the water. The temperature was between 22 to 26°C.

1. LOCAL MECHANICAL STIMULI.

The local stimulus was applied to the animal by the use of a glass capillary. The reactions are summarised in Table 6.

a) The tentacles stimulated by a gentle touch responded with a slight retraction of the touched tentacles. A stronger touch produced the retraction of all the tentacles; a more severe touch led to the retraction of the tentacles and the anterior part of the trunk. Even by severe or injurious treatment no remarkable change occurred in any part of the body except the anterior.

TABLE   6.

Response to mechanical stimulus on the various parts of the animal.
Number of animals tested 17.

Stimulus \ Part stimulated	Tentacles	Genital Papilla	Trunk, anterior	Trunk, middle	Tail
Light touch	withdrawal of the stimulated tentacle	withdrawal of the tentacles	withdrawal of the tentacles and the anterior trunk	local contraction	slight shortening.
Gentle touch	retraction of the tentacles and the anterior trunk	same as left	same as left	same as above	same as above
Strong touch	same as above, response much increased	same as left	same as left	same as above, response much increased	shortening of the whole tail

b) The genital papilla is situated on the dorsal surface close to the oral brim. The mechanical stimulation of the genital papilla called forth, besides a slight shortening of itself, response in the tentacles and the anterior part of the trunk. The relation between the intensity of stimulus and the magnitude of reaction was the same as in the tentacles.

c) The entire body surface was very receptive to mechanical stimuli; by a weak stimulation a ring like depression occurred in the part stimulated, the opposite part, however, was little affected. If the stimulus was sufficiently strong, besides the local constriction, a remarkable shortening of the longitudinal muscles took place locally. These local constrictions showed no sign of propagating others elsewhere in the body, provided that the stimulus applied was not too abnormally strong. In the anterior part of the trunk the retraction of the tentacles and the anterior trunk accompanied the local contraction. A slight touch of the oral brim led to the withdrawal of the tentacles. The region of the body which was able to induce the response of the tentacles and the anterior trunk by a gentle mechanical stimulus was restricted to the region within one centimetre from the oral brim.

d) The tail. Owing to the extreme contraction of the whole tail, the response of the circular muscles was difficult to observe, but the shortening of the longitudinal muscles was easily observed. The magnitude of the reaction was proportional to the intensity of the stimulus. To a strong mechanical stimulus, the entire tail and the posterior region of the trunk fell into the state of contraction, but nothing was remarked in the tentacles or anterior half of the trunk.

On repeated weak stimulation a summation of the local contraction occurred in any part of the body stimulated. On the middle part of the body a deep furrow was formed at the spot stimulated, and on rapidly repeating the stimulation, this furrow spread to the opposite side of the body, and a ring-like depression, deepest at the stimulated spot, was formed.

The amputation of the anterior or the posterior end of the body called forth severe contraction of the injured portion. In both cases the contraction was restricted to the vicinity of the injured portion, the opposite half of the body remaining unchanged, provided that

any direct disturbance of the body was avoided by careful treatment.

2. STIMULATION OF THE ENTIRE BODY SURFACE.

To general mechanical stimuli, such as jarring the water, or dropping water on the surface of the aquarium water, or tapping the wall of the aquarium, the animal responded with the tentacles and the anterior trunk. A very weak stimulus caused the tentacles to stop the movement or to retract slightly. To a stronger one, both the tentacles and the anterior trunk reacted. The reactions to the disturbance of the water were less severe than those to local mechanical stimulation.

3. MUSCLE TONUS AND MECHANICAL STIMULI.

When we dug up the sand and pulled the animal out of the sand by seizing the anterior portion of the trunk, the whole body of the animal was soft with an enormous length, showing no remarkable distinction between the tail and the trunk. But after a few seconds

TABLE 7.

Body length of the animal in a natural habitat and in contraction after
it is dragged out of sand. Temperature 22.5°C.

Body weight without sand, in grams	Body length, natural, in cms.			Body length contracted, in cms.			Ratio		
							tail / trunk		total length, natural
	trunk	tail	total length	trunk	tail	total length	natural	con-tracted	Same contracted
15.57	8	15	23	5.5	3.8	9.3	1.9	0.69	2.5
17.88	12	22	34	6.7	4.2	10.9	1.8	0.63	3.1
18.97	10	20	30	7.7	5.5	13.2	2.0	0.71	2.3
25.96	12	21	33	7.8	4.8	12.6	1.8	0.62	2.6
27.62	12	22	34	8.6	4.9	13.5	1.8	0.57	2.5
30.30	10	18	28	9.6	5.2	14.8	1.8	0.54	1.9
31.60	10	20	30	7.7	6.0	13.7	2.0	0.78	2.2
33.57	13	23	36	8.7	5.4	14.1	1.8	0.62	2.6
35.29	11	25	36	7.7	4.0	11.7	2.3	0.52	3.1
35.69	11	18	29	8.3	4.9	13.2	1.6	0.59	2.2
38.61	10	16	26	8.2	6.0	14.2	1.6	0.73	1.8
40.40	11	19	30	9.5	6.2	15.7	1.7	0.65	1.9
Mean							1.8	0.64	2.4

all the musculatures gained definite tonus and the animal assumed a definite shape and hardness, the tail becoming distinct from the trunk. As a result of the sudden increase in muscular tonus, the body increased in width and decreased in length. The approximate length measured immediately after the animal was drawn out of the sand was designated as its natural length. This and the length in contraction, and also the ratio of these lengths, are given in Table 7.

The form of the body is determind by the tonic relations among circular and longitudinal muscles, the elastic fibres in the subepithelial connective tissue (JORDAN 1914, UEXKÜLL 1926) and the pressure of the body fluid. Table 7 indicates decidedly the increased tonus of the muscles because of the absence of the continued mechanical stimulation (Das Nichtberührtsein in the term of v. BUDDENBROCK 1924, p. 98) of the entire surface by the sand. The continuous mechanical stimulus by sand seems to act on the animal in decreasing tonus. The following observation confirms this interpretation: when the animal was placed in water, irregular constriction due to the ununiform tonic contraction of the circular muscles appeared on the upper body surface, while on the contrary, the lower surface which was in contact with any substratum showed much less constriction and seemed rather smooth; and on alternating the upper side with the lower, the same effect always was brought about. Such disapperance of irregular constriction and the decrease in muscle tonus were also found on reexamination of the animals after they had burrowed into the sand. The tonus-decreasing action was independent of the size of the sand grains, such that the following results were found : —

Aug. 26, 1927.		4. 20 P. M.	
	A	B	C
	in fine sand	in coarse sand	in gravel and small pebbles
after 30 mins.	the body was elongated and became smooth	same as A	same as A
number of animal	2	2	2

Aug. 23.		4. 15 P. M.	
	A	B	C
	in coarse sand	in coarse sand	in fine sand
after 30 mins.	same as A on Aug. 26	same as A	same as A
number of animals	2 (young)	2 (old)	2

The hardness of the body was markedly increased by treatment such as rubbing the entire surface with a cloth or with the hand, or dropping the animal on the ground from a height of a few centimetres.

4. DISCUSSION.

For comparison of the responses of the various parts of the body, the magnitude of the reaction must be considered. The reaction time was so short that an accurate measurement was impossible even with a stop watch. The magnitude of the reaction was in the following order (in decreasing series): anterior part of the trunk>tail>middle part of the trunk. In both ends of the body the contraction of the longitudinal muscles was greater than that of the circular muscles. The facility to give rise to a reaction of the tentacles and the anterior end on stimulating the various parts of the body was in the following order : — oral brim>genital papilla>tentacles>anterior part of the trunk. The reactions to mechanical stimulation were in general local, if the stimulus were not too strong. With a strong stimulus, the range and magnitude of the reaction far increased, yet remaining local. The reactions were in general to avoid and protect the body from the injurious stimuli.

The order of responsiveness in *Caudina* is the same as in *Holothuria* (CROZIER 1915 b, p. 255) and many other animals, except for the tentacles. Distant reaction is developed in the anterior end of the body, but it is of a far lower degree in comparison with that of *Chromodoris* (CROZIER and AREY 1919).

In *Caudina* the ratio of the natural and the contracted body lengths is 2.4 in an average value (TABLE 7). CROZIER (1915 b) observed the change of the body length of *Holothuria* under mechanical stimulus, as follows :

No.	normal	contracted	normal contracted *
1	15.5	10.0	1.6
2	10.0	4.0	2.5
3	16.5	8.9	1.8
4	19.1	9.0	2.6
5	18.0	8.6	2.1
		Mean	2.1

(* the last column was calculated by the author).

According to PEARSE ('08) the length of *Thyone* is 20 cms. in a fully extended animal and 6–7 cms. in a contracted one. In this case, extended/contracted is 2.8–3.3. In these animals the degrees of contractility are not far different; the higher degree in *Caudina* and *Thyone* than in *Holothuria* seems to be due to their sand inhabitance.

JORDAN (1914 p. 423) attributes the hardening of the holothuria by a gross mechanical stimulus to the tonic contraction of the muscle-like fibres in the connective tissue, and later v. UEXKULL (1926) has shown that this tonic contraction occurred with absence of the sensory cells. The state of the hardening of the body under gross mechanical stimulation is distinguished from that of the tonic contraction of both the musculatures by the hardness of the body wall and not from the shape of the animal. If there were no other fibres besides the elastic fibres in the tegument of *Caudina*, the hardening of the body wall would probably be brought about by the tonic contraction of the fibres in question (TAO 1927 p. 266).

The entire surface of *Caudina* is very receptive to mechanical stimuli and the responses are quite adaptable to its life in the sand. The relaxed state of the musculatures of the entire body in the sand is the most favourable state for reaction to external stimuli (UEXKULL 1921, p. 54).

III. GEOTROPISM.

The posture of *Caudina* in the sand is described in detail in my previous paper. Let us repeat the description briefly, for convenience in further explanation. The extremely elongated body of the animal makes about 30° inclination to the surface of the sea bottom, while the tip end of the tail lies just on the bottom surface directed ordi-

narily perpendicularly. The dorsoventrality is maintained as in the common holothuria, that is, we find the trivium on the lower side and the genital papilla on the upper surface (YAMANOUCHI 1926, Fig. 1). The existence of the positive geotropism in the anterior trunk was proved in the previous paper. In this chapter the geotropism found in the posterior part of the body and the problem of the geotropism of this animal will be discussed.

1. NEGATIVE GEOTROPISM OF THE TAIL.

While this animal was placed in the sea water, the trunk bent in any direction, due to the differences of tonus among the five pairs of longitudinal muscles. The tail was found in many cases bending more or less upwards. In such a case, if we reverse the position along the main axis, the tail bent again upwards and remained unchanged, and the anterior end bent somewhat downwards, as indicating the natural posture in the sand. To determine the localization

TABLE 8.

Time in which the tip end of the tail emerges on the bottom surface
after the animal is imbedded in sand.

Mode of operation	Number of animals	Depth imbedded in cms.	Time of reapperance in minutes					Mean in minutes
Normal (unoperated)	5	7	14,	17,	22,	23,	30	21.2
Without the nerve ring	5	7	13,	18,	20,	28,	36	23.0
Without the tip end of the tail	5	7	18,	20,	30,	31,	37	27.2
Without half of the tail	5	7	25,	35,	41,	55,	64	44.0
Without the whole of the tail	5	7	82,	107,	155,	160,	161	133.0
Normal (unoperated)	5	14	39,	45,	45,	65,	68	52.4
Without the nerve ring	5	14	40,	50,	50,	60,	145	69.0
Without the tip end of the tail	5	14	75,	80,	110,	130,	160	111.0
Without the whole of trunk (only the tail)	5	3	15,	15,	58,	73,	85	49.2
„	5	5	56,	120,	130,	172,	240	143.6

of this negative geotropism the following experiments were undertaken.

Normal (intact animals for control) and operated animals were embedded in the sand at depths of 3, 5, 7 and 14 cms., and the time (in minutes) of their emergence on the surface was measured. The part which first reappeared on the surface was always the tail region of the body. The isolated tail could reappear on the surface as well as the operated animals.

Table 8 indicates that (1) the negative geotropism of the tail is independent of the nerve ring, (2) the negative geotropism is localized in the tail region, (3) it is strongest in the tip end and decreases anteriorly along the tail, (4) the isolated tails behave just the same as the intact animals, (5) the time of emergence increases by increasing the depth imbedded, (6) this increase in time is more than the increase in depth and (7) this increase is especially marked in operated animals, most of all in the isolated tail.

The reaction was accomplished mainly by the elongation of the tail, the trunk remained unchanged at the initial situation. The tail end reappeared on the surface ordinarily a few centimetres (1—3.5) forward from the point just above the initial situation or a few centimetres backward from the direction above mentioned (Fig. 3).
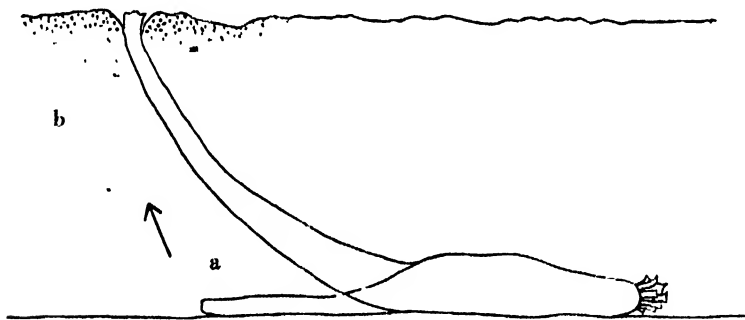
Fig. 3. Negative geotropism of the tail.
a: initial situation placed on the bottom.
b: responded situation.

The parts of the tail which first appeared on the surface were in most cases the end of the tail (the cloacal papilla or the cut end),

in fewer cases, another part of the tail; the result of examination of
17 isolated tails being:

first appeared the tip end   10 cases

         ,,   the base   1 case

and   ,,   the part between both ends   6 cases.

When any part except the tip end first reappeared the tail regained
the ordinary posture by the normal postero-anteriorly directed rhythmic
movements of both the longitudinal and the circular muscles (Fig. 4).
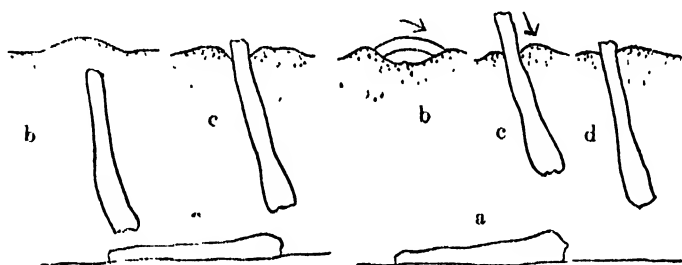
Fig. 4. Negative geotropism of the tail.
a — c and a — d indicates the successive position of the tail.

2. DISCUSSION.

The responses of *Caudina* to gravitation are of three kinds, the
positive geotropism of the anterior trunk, the righting reaction (YAMA-
NOUCHI 1926), and the negative geotropism of the tail. The negative
geotropism of the tail is independent of the presence of the trunk.
Although this animal has five pairs of statocysts filled with numerous
granules close to the nerve ring (private communication of Mr. KAWA-
MOTO), its true function is not known, owing to the difficulty of
removing it. VON BUDDENBROCK proved that the positive geotropism
of *Arenicola grubei* disappeared by operation on its statocysts; he
also reported many cases of positive getropism in sand-dwelling animals,
Arenicola grubei, A. mariana, Synapta, Solen, Branchioma, Convoluta
and *Myxicola* (BUDDENBROCK 1912, 1913); according to him all these
animals possess statocysts. From his experiment on *Arenicola* it is
clear that the statocyst is the receptor for gravitational force. But
how can animals possessing only positive geotropism stay at a definite

depth ? In *Caudina* the natural posture is maintained by three components, and we certainly need not assume any unknown factor, such as Hemmung des Einbohrreflexes (BUDDENBROCK 1913). The positive geotropism of *Caudina* may perhaps be due to the presence of statocysts as in many other sand dwelling animals. But in the tail region we cannot find any special receptor, so that the impulse from a statocyst is not a question in this case (the anatomy of *Caudina arenata* was investigated by GEROULD 1896, *C. chilensis* by KAWAMOTO 1927). The receptor of the righting reaction of an echinoderm is totally obscure as indicated by WOLF (1925).

According to OLMSTED (1917), *Synaptula* shows definite orientation to gravity, and the same is true of *Cucumaria* (LOEB 1918), but *Thyone* (PEARSE 1908) and *Holothuria* (CROZIER 1915 b) show no definite orientation to gravity. In the instance of *Caudina*, geotropism is the most important factor in the maintenance of its life, the positive geotropism being the essential factor for food-taking, and the negative geotropism for respiration. The last factor is especially important from an ecological viewpoint. As the dwelling place of *Caudina* is shallow sea bottom, the sand is frequently disturbed by storms, especially at low tide. We find however, the tip end always normally pulsating on the surface of the sea bottom due to the negative geotropism of the tail.

IV. CHEMICAL STIMULI.

The reactions of invertebrate animals to chemical stimuli have been hitherto studied by many authors, but little attention is paid to quantitave studies, except by a few authors. It is also a most important problem in animal physiology to call attention to individual differences. In this investigation these points are specially considered in the measurement of the reaction time, and of the threshold value.

1. METHOD.

Animals to be studied were placed on a cloth in a glass jar as in the instance of mechanical stimulation. The part to be stimulated was exposed to the air and about half a c.cm. of the solution was carefully applied upon it by a glass pipette. After the stimulation

the whole body was immersed in sea water. The part once stimulated
was avoided as principle in further stimulation, especially in the
measurement of reaction time. In such a case as the determination
of threshold values (tables 10, 12, 13 and 15), when the number of
stimulations was increased, the same part was stimulated more than
once. In the latter case the appearance of the body surface after
the stimulation was noticed, and the stimulation was further accom-
plished only when the part in question returned to the initial appear-
ance after each stimulation. The intervals of the successive stimula-
tions were about 3 minutes. Chemicals used were extra pure prepar-
ations of MERCK and KAHLBAUM. Necessary caution was used for
their purity.

As distilled water brought about no reaction when locally applied,
most of the reagents were dissolved in it, except chloroform, ether,
and menthol, which were saturated in sea water. Any required con-
centration was prepared before the experiment by dilution of the stock
solution with distilled water. The reaction times and the threshold
values for chemical stimuli were measured. As to the threshold value
in this investigation, it signifies the smallest limit of concentration
which called forth any definite response, however small its magnitude
in the animal. The temperature of sea water changed from 22 to
27°C during the investigation. But the change of temperature in each
experiment was neglegibly small.

2. REACTIONS OBTAINED.

The entire body surface of the animal was receptive to chemical
stimuli. Reaction was in most cases local contraction of the part
stimulated. The part stimulated with alcohol or other narcotics as-
sumed a different appearance from the ordinary cases (part f in this
chapter). With an increases in concentration, the reaction time
decreased, while the magnitude became larger and the responses occur-
red in a wider range. If the stimulus was too strong, the local
constriction spread in an anterior or posterior direction as a peristaltic
wave, ordinarily in the former direction. In the anterior part of the
trunk the complete retraction took place on stimulation. Generally
the reaction was the most obvious in the middle portion of the trunk,

so in the following description it means that the middle part of the trunk was stimulated if not specially noted.

a) Osmotic stimulus. Distilled water gave no reaction in any part of the body when locally applied; hypotonic sea water solution was also non-effective, except in the case when it contained some stimulating ions. On the other hand, *Caudina* was neither very receptive for hypertonic sea water solution. The sea water concentrated to 2/3 of its original volume gave distinct local reaction. Glycerol above a 3 molar solution had a stimulating action (Table 9).

TABLE 9.

Osmotic stimulation by the concentrated solution of glycerol. Temp. 23–24°C.

No. of animal	Threshold value in M.*	Reaction time in sec.	
		7 M.	10 M.
3	3		
4	4	3.7	3.5
5	5	4.3	4.2
6	4	5.1	5.3
7	4	4.3	4.4
8	3	5.2	4.8
9	3	3.9	3.9
10	4	4.1	3.9
	Mean	4.37	4.29

*M means a molar solution in this report.

The stimulation by concentrated sea water at the concentration above mentioned is not merely osmotic but ionic. The last factor may be predominant, for the changes of ionic compositions by evaporation exert additional effect, as pointed out by CROZIER (1915 b, p. 285). The stimulation of concentrated glycerol solution is osmotic as indicated by the reaction times.

b) Salts. The limiting concentration of four alkaline chlorides is given in Table 10. The wide differences of the threshold concentrations among the four salts are due to the nature of the cations. At the concentration of 1.0 M, NaCl and LiCl do not stimulate osmotically, but as ionic stimuli, for glycerol acts as an osmotic stimulus above a 3 molar solution. The order of stimulating effects of Ammonium and

TABLE 10.

Four Chlorides. Threshold value in M.

Body weight without sand, in grams	NH_4Cl	KCl	NaCl	LiCl
38.10	0.1	0.1	0.5	—
29.54	0.1	0.1	0.6	0.7
48.52*	0.01	0.01	0.4	0.5
32.58	0.1	0.1	—	—
—	0.1	0.1	—	—
—	—	—	1.0	1.0
—	—	—	0.7	0.9
35.94	—	—	0.8	1.0
36.08	—	—	1.0	1.0
Range	0.01–0.1	0.01–0.1	0.4–1.0	0.5–1.0
Mean	0.1	0.1	0.77	0.92

*This animal is omitted from the mean.

Potassium Chlorides was determined by the measurement of reaction times (Table 11).

TABLE 11.

NH_4Cl and KCl. Number of animals tested 12.

	Concentration	Reaction times in sec.	
		Range	Mean
KCl	0.3 M	1.5–2.7	1.94
NH_4Cl	0.3 M	1.5–2.7	1.97

The dissociation degrees of these salts at 18°C are:

	KCl	NH_4Cl	NaCl	LiCl
0.1 M	0.861(1)	0.851(1)	—	—
0.3 M	0.810(1)	0.801(1)	—	—
0.5 M	—	—	0.773(2)	0.766(2)
1.0 M	—	—	0.745(3)	0.735(3)

(1) Calculated from the equivalent conductivities in Landolt-Bernstein's Tabellen.

(2) Cited from Lewis' System of physical Chemistry, Vol. I.

(3) Calculated as (1), taking the viscosity of solution into consideration.

The difference between threshold values of KCl and NaCl cannot be explained by the difference of the dissociation degrees. This is due to the specific action of the cations. From Tables 10, 11 and above table, the order of stimulating power of the four salts is in decreasing series as follows:

$$KCl, NH_4Cl > NaCl > LiCl.$$
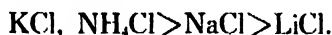
Binary salts such as $CaCl_2$, $MgCl_2$ and $SrCl_2$ in concentration of 0.35 M and $MgSO_4$ in 1.0 M solution were not stimulating. While $BaCl_2$ was strongly stimulating; the threshold concentration obtained from 8 animals was within 0.02 and 0.1 M.

Anions. As the Potassium salts were a good stimulant, five salts of Potassium were tested (Table 12). The dissociation degrees of these

TABLE 12.

Potassium salts. Threshold value in M.

Body weight without sand in grams	K_2SO_4	KBr	KI	KCl	KNO₃
35.05	0.1 (5)	0.12(1)	0.13(3)	0.14(4)	0.15(2)
43.45	0.1 (1)	0.15(3)	—	0.15(2)	0.15(4)
34.62	0.08(2)	0.13(4)	--	0.15(1)	0.17(3)
34.86	0.08(2)	0.12(1)	--	—	—
58.20	0.07(1)	0.11(3)	0.13(4)	0.11(2)	—
29.04	—	0.11(4)	0.11(3)	0.11(2)	0.15(1)
32.58	0.08(3)	—	0.11(1)	0.11(2)	--
32.87*	—	—	—	0.04(2)	0.06(1)
Mean	0.085	0.12	0.12	0.13	0.16

*This animal is omitted from the mean.
Number in parenthesis is the order of stimulation.

salts at 18°C (calculated from the equivalent conductivities in LANDOLT-BERNSTEIN's Tabellen) and their reciprocals are as follows:

	½ K_2SO_4	KBr	KI	KCl	KNO₃
0.1 M	0.715	0.863	0.869	0.861	0.828
reciprocal × 0.112	0.078 *	0.130	0.129	0.130	0.135

* The reciprocal number is divided by two.

Comparing the mean values given in Table 12 with the above table, it is evident that the amount of the active ions is the important factor

for stimulation. It seems that the stimulating power of KNO_3 and K_2SO_4 is less than that of the other three salts. The effect of anions was further compared in Sodium salts (Table 13). It is without doubt that the anions also play an important role in stimulation.

TABLE   13.

Sodium salts.   Threshold value in M.   Number of animals tested 7.   Temp. 25-26°C.

Salts	Range	Mean
Formate	0.5 m no reaction	
Acetate	the same	
Sulphate	the same	
Citrate	0.15-0.3	0.22
HPO_4	0.15-0.25	0.21
Cl*	0.4 -1.0	0.77

*quoted from Table 10.

TABLE   14.

Potassium Chloride.

Body weight without sand, in grams	Threshold volue in M.	Temperature °C.
48.52	0.01	25
32.87	0.04	26
—	0.05	25
—	0.1	26
35.01	0.1	22
26.75	0.1	,,
63.34	0.1	,,
53.52	0.1	24
—	0.1	23
58.20	0.11	—
29.04	0.11	—
32.58	0.11	26
35.05	0.14	—
37.33	0.14	—
43.45	0.15	—
34.62	0.15	—
Mean	0.10	

In these and further experiments the animals tested were all adult individuals in their appearance and body weight. The differences in temperature were not very small, but this is not the chief cause of the individual differences as indicated by Table 14. If necessary caution is paid, and the investigator is well acquainted with the animal, the mean value is reliable as a basis for interpretation. The mean value shown in Table 14 is just same as that given in Table 10. When the animals tested were small in number, it was of course necessary to be careful in averaging the experimental results.

c) *Antagonism of ions.* The phenomenon of antagonism of ions has been hitherto known in vast fields of biological phenomena, but studies on it in the physiology of the receptor organ are not abundant. In *Caudina*, calcium ions inhibited the stimulation of monovalent ions such as Na and NH_4 (Table 15), while in themselves unable to stimulate. In some individuals complete inhibition was not observed even by a high concentration of calcium ions (more than 5 in 100 parts by volume). But even in such a case the magnitude of reaction was more reduced than in the case without calcium ions. In this instance, the antogonism was of course existing, and was simply incomplete.

TABLE   15.

Antagonism of ions.   Minimum concentration (by volume) of Calcium Chloride that inhibits the stimulation of Natrium and Ammonium Chlorides.   Temp. 25–26.5°C.

	NaCl	NH_4Cl	$CaCl_2$		Number of cases	Remarks
	1.0 M	0.5 M	1.0 M	0.5 M		
100 parts by volume consisting of	99		1		1	
	98		2		2	
	97		3		4	
	95		5		1	
	95		5		3	no antagonism
		98		2	2	
		97		3	3	
		96		4	3	
		95		5	1	
		95		5	2	no antagonism

The relations of Ca and Mg to the monovalent ions in antagonistic action are as follows (+ or − means respectively the ·presence or absence of the antagonistic action).

	NH_4Cl	KCl	NaCl	LiCl
$CaCl_2$	+	−	+	−
$MgCl_2$	−	−	−	−

d) *Acid and Alkali.* Acid and alkali were very effective stimulants even in very diluted solutions (Tables 16 and 17). Individual differences were remarkable in strong acids. The limiting concentrations are roughly inversely proportional to the dissociation degrees, but the weaker acids or alkali are more effective than the stronger ones if the dissociation degrees are taken into consideration.

<div align="center">TABLE 16.</div>

<div align="center">Acid. Threshold value in normal.</div>

Acid	Number of animals	Range	Mean
Trichloroacetic	7	0.0002–0.004	0.001
Hydrochloric	7	0.0003–0.0035	0.001
Sulphuric	6	0.0004–0.002	0.001
Oxalic	6	0.004 –0.01	0.0065
Formic	7	0.006 –0.01	0.008
Acetic	10	0.01 –0.05	0.03
Butylic	9	0.02 –0.05	0.04
Picric	2	0.005	0.005

<div align="center">TABLE 17.</div>

<div align="center">Alkali. Threshold value in normal.</div>

	Number of animals	Range	Mean
NaOH	6	0.002–0.008	0.006
NH_4OH	5	0.003–0.01	0.008

To ascertain this point, solutions of acids and alkalies each containing an equal quantity of hydrogen ions were prepared, such that

pH	Standard		Indicator
2.4	1.0 normal	acetic acid	Thymol-blue
2.9	0.1 n	„	Brom-phenol-blue
11.8	1.0 n	ammonia	Alizaringelb R

(pH values are cited from MICHAELIS 1922, p. 21).

TABLE 18.

Acid and alkali. Number of animals tested 7. Temp. 26-27°C.

pH	Acid or alkali	Reaction time in sec.		No-reaction cases
		Range	Mean	
2.9	HCl	4.0-5.3	4.74	2
	HCOOH	3.1-4.4	3.75	1
	CH₃COOH	2.7-4.4	3.65	1
2.4	HCl	2.8-4.8	3.63	1
	HCOOH	1.8-3.2	2.38	0
	CH₃COOH	1.8-2.3	2.00	0
11.8	NaOH	3.0-5.8	4.47	1
	NH₄OH	1.2-2.3	1.65	0

In acids or alkalies, with equal pH value, the fact that the weaker ones are more effective than the stronger ones is decidedly shown in reaction times, number of no reaction cases, and in the magnitude of reaction.

TABLE 19.

Alkaloid.

Alkaloid	Number of animals	Reaction
Quinine Hydrochloride	3	0.001 -0.005 M reactive
Strychnine Nitrate	4	0.001 -0.01 M reactive
Nicotine	4	0.0001-0.001 M reactive
Atropine Sulphate	16	0.02 M non-reactive
Caffeine	16	the same
Cocaine Hydrochloride	15	the same
Pilocarpine Hydrochloride	16	the same
Morphine Sulphate	5	the same

pH	Standard		Indicator
2.4	1.0 normal	acetic acid	Thymol-blue
2.9	0.1 n	„	Brom-phenol-blue
11.8	1.0 n	ammonia	Alizaringelb R

(pH values are cited from MICHAELIS 1922, p. 21).

TABLE 18.

Acid and alkali. Number of animals tested 7. Temp. 26-27°C.

pH	Acid or alkali	Reaction time in sec.		No-reaction cases
		Range	Mean	
2.9	HCl	4.0-5.3	4.74	2
	HCOOH	3.1-4.4	3.75	1
	CH₃COOH	2.7-4.4	3.65	1
2.4	HCl	2.8-4.8	3.63	1
	HCOOH	1.8-3.2	2.38	0
	CH₃COOH	1.8-2.3	2.00	0
11.8	NaOH	3.0-5.8	4.47	1
	NH₄OH	1.2-2.3	1.65	0

In acids or alkalies, with equal pH value, the fact that the weaker ones are more effective than the stronger ones is decidedly shown in reaction times, number of no reaction cases, and in the magnitude of reaction.

TABLE 19.

Alkaloid.

Alkaloid	Number of animals	Reaction
Quinine Hydrochloride	3	0.001 -0.005 M reactive
Strychnine Nitrate	4	0.001 -0.01 M reactive
Nicotine	4	0.0001-0.001 M reactive
Atropine Sulphate	16	0.02 M non-reactive
Caffeine	16	the same
Cocaine Hydrochloride	15	the same
Pilocarpine Hydrochloride	16	the same
Morphine Sulphate	5	the same

e) Alkaloid. Among eight alkaloids tested, quinine, strychnine and nicotine were able to call forth local contraction. Nicotine was the most effective among the three (Table 19).

f) Narcotics. Alcohols, ether and chloroform were applied. Body surface stimulated by these narcotics exhibited small constriction and numerous fine crinkles different from the distinct local contraction brought about by salts or other stimulants. Under phenol and menthol, the reactions were more distinct than with other reagents. In the aliphatic alcohols the stimulating efficiency is almost doubled with one increase in CH_2. The order of stimulating power is as follows (in decreasing series) : —

menthol>phenol>butyl alc.>propyl alc.>
ethyl alc.>methyl alc.

TABLE   20.

Narcotics.

	Concentration in M.	Number of animals	Reaction	
			Reactive	not reactive
Methyl alcohol	10.0	11	7	4
	7.5	10	4	6
	5.0	14	1	13
Ethyl alcohol	10.0	12	11	1
	7.5	10	8	2
	5.0	14	6	8
Propyl alcohol	5.0	16	14	2
	2.5	11	9	2
	1.0	10	4	6
Butyl alcohol	0.4	13	5	8
Phenol	0.5	2	2	—
Menthol *	saturated	4	4	—
Ether *	saturated	15	9	6
Chloroform *	saturated	14	12	2

*Saturated in sea water.

g) Sugar. Cane sugar in 1 molar solution and maltose in half molar solution were not effective. The same was true with saccharine in 0.1 molar solution.

h) Effect of dilution. With dilution of the solution the magnitude

NOTES ON THE BEHAVIOR OF THE HOLOTHURIAN

TABLE 21.

Effect of dilution. Temp. 24–25°C.

	Molar dilution	Reaction time in sec.		Number of animals
		Range	Mean	
KCl	1	1.3–1.6	1.43	10
	2	1.5–2.1	1.66	
	5	1.9–3.9	2.53*	
	10	2.1–4.4	3.25**	
HCl	10	1.5–1.7	1.58	7
	20	1.8–2.8	1.98	
	100	2.5–3.5	2.91	
	200	3.2 4.6	3.88	

*With one no reaction case.
**With two no reaction cases.

diminished and the reaction time increased. The deviation in reaction time also increased with dilution in either case.

i) Part of the body. In mechanical stimulation the anterior trunk was the most receptive part of the body; it is also true in the case of chemical stimulation. From the tables 22 and 23, it is clear

TABLE 22.

Reaction time in seconds. Temp. 23–24°C.

Part of the trunk stimulated	KCl 0.4 м		HCl 0.025 N.	
	Range	Mean	Range	Mean
Anterior	1.8–2.6	2.10	1.4–4.4	2.76
1/3 from anterior	1.8–3.2	2.40	1.6–5.8	3.52*
2/3 from anterior	1.8–3.2	2.38	1.4–4.8	3.30
Posterior	2.0–3.4	2.50	2.4–4.8	3.52
Number of animals	10		10	

*The mean value became too high as the result of two extraordinary reaction times, 5.4 and 5.8 seconds.

that the reactiveness for chemical stimuli decreases in the following order : —

anterior part of trunk>middle part of trunk>posterior part of trunk.

The tail region was in so severe contraction that an accurate measurment of reaction time could not be accomplished with ease.

j) Nerve ring. The animal whose nerve ring was removed by the amputation of the anterior end of the trunk reacted almost the same as before the amputation.

TABLE 23.

Reaction time in seconds. 0.025 N HCl.
A. Before the amputation of the anterior part of the trunk.
B. After its amputation.

Body weight without sand, in grams	A. with nerve ring			B. without nerve ring			Temp. °C
	Trunk			Trunk			
	anterior	middle	posterior	anterior	middle	posterior	
--	1.9	2.5	2.1	2.1	2.9	2.4	25
—	3.0	2.6	3.5	3.5	3.1	3.4	,,
--	3.0	3.0	3.7	3.4	3.6	3.6	,,
39.85	2.7	3.6	3.1	2.8	3.5	3.0	24
34.22	2.6	3.1	2.8	3.0	3.3	4.4	,,
34.03	2.8	2.6	3.1	3.8	3.0	3.3	,,
48.31	2.2	2.7	2.9	2.3	3.2	3.6	,,
54.32	3.9	3.1	4.3	3.4	3.4	3.8	,,
46.09	2.9	3.3	3.2	3.2	2.9	3.1	,,
49.07	2.4	3.3	3.6	2.4	3.1	3.6	,,
Mean	2.74	2.98	3.23	2.99	3.20	3.42	

The differences in reaction time before and after the amputation are (from Table 23.) : —

	anterior	middle	posterior
before	2.74	2.98	3.23
after	2.99	3.20	3.42
difference	0.25	0.22	0.19 seconds.

The increase in reaction times after the amputation may be due to the shock effect of the amputation, for the increase is largest at the anterior part. It should be noted that the order of responsiveness in three parts remained unchanged.

k) Age. The responsiveness to chemical stimuli changes with the

body weight of the animal (Table 24), highest in the animals of
10–20 grams, and was in the following order : —

10–20 > 0–10 > 20–30 > 30–50 grams.

TABLE 24.

The relation of the body weight and the reaction time for the
chemical stimulus. Temp. 24.5–27°C.

Body weight without sand in grams	Number of animals	Mean reaction time in sec.			
		KCl		HCl	
		0.2 M	0.4 M	0.01 N	0.02 N
0–10	5	2.40	1.68	2.48	1.78
10–20	5	2.30	1.58	2.40	1.86
20–30	5	2.32	1.78	2.70	2.02
30–50	6	2.94*	1.70	3.14**	1.98

*With two non-reaction cases.
**With one non-reaction case.

3. DISCUSSION.

The process of stimulation by high osmotic pressure is different
from the case of other stimulants, for the reaction time is much
longer, roughly corresponding to that of diluted acid or alkali (com-
pare Table 9 with Tables 18 and 21), and is little affected by
dilution. *Caudina* was not reactive to hypotonic solutions, including
distilled water, and was reactive to hypertonic solutions which gave
more than twice the depression of the freezing point of their environ-
ment. According to CROZIER (1915 b) *Holothuria* are non-reactive
both to hyper- and hypotonic solutions. *Synaptula* (OLMSTED 1917),
Ascidia (HECHT 1918 a) and *Chiton* (AREY and CROZIER 1919) are
reactive to both solutions. CROZIER (1915 b, p. 284) is of the opinion
that as *H. surinamensis* lives in the shallow sea the insensitivity is
adapted to its environment. It is also true in the case of *Caudina*,
as it lives in the shallow sea bottom. The reactions to substances
giving a sweet sensation to the human taste are not the same among
animals. In the next table the numbers represent the concentration

of the solution in mols, and + or − means respectively reactive and
non-reactive cases,

	Cane sugar	Maltose	Lactose	Solvent	Author
Holothuria	2−	0.1+		rain water	CROZIER '15 b
Synaptula	0.5+			sea water	OLMSTED '17
Caudina	1−	0.5−		dist. water	
Chiton	0.33+	0.33−	0.5+	sea water	AREY & CROZ. '19
Chromodoris	1−	1−	satur.−	dist. water	CROZ. & AREY '19
Ascidia	1−			dist. water	HECHT '18 a

In *Synaptula*, the stimulation seems to be osmotic, for glycerin gives
the same effect (OLMSTED 1917). According to CROZIER, *Holothuria*
also give response to 0.1 M glycerin, and OLMSTED found that *Synaptula*
is reactive to 0.005 M saccharine whereas it gives no reaction to sugar
and glycerin. *Caudina* was non-reactive to these substances. On
the whole, it is in rather rare cases that aquatic animals are found
reactive to substances which taste sweet to human beings.

The stimulation by neutral salts is chiefly dependent upon cations.
The order of stimulating power of the chlorides to the chemo-receptors
of many animals is given in the following table. The cations are

	Method	Concentration in M	Order	Author
Holothuria	magnitude, react. time	0.1	$K>Na>Li>NH_4$	CROZIER '15 b
Synaptula	limit. conc.		$K>NH_4>Li$	OLMSTED '17
Caudina	limit. conc. react. time		$K, NH_4>Na>Li$	
Ascidia	limit. conc.		$K>NH_4>Na$	HECHT '18 a
Chiton	magnitude	0.625	$K>NH_4>Li>Na$	AREY & CROZIER '19
Chromodoris	,,	,,	$K>NH_4, Li>Na$	CROZIER & AREY '19
Allolobophora	react. time	0.1–0.002	$Na>NH_4>Li>K$	PARK.& METCALF '06
Helodrilus	,,	0.02	$K>NH_4>Na>Li$	,,
Rana	,,	0.5–3.0	$NH_4>K>Na>Li$	COLE '10
,,	,,	0.1–2.0	$K>Na$	CROZIER '15 a
,,	,,	1.0	$K>Na, Li$	GRÜTZNER '94
Homo	intensity, react. time	1.0	$K>Na>Li$	,,

arranged in the decreasing order of stimulating power. In the last two experiments of GRÜTZNER stimulation was made by the direct application of chemicals to the nerve fibres. NH_4 and K are as a whole more effective than Na and Li. It is explained in part by the differences of the dissociation degrees. But the phenomena found in *Holothuria*, *Chromodoris* and *Allolobophora* are quite difficult to account for by this explanation. Whereas KCl and NH_4Cl have almost equal dissociation degrees, K is in most cases more stimulating than NH_4; and Li in some cases surpasses Na; in *Caudina*, *Helodrilus* and *Rana* the orders are nearly coincident with the dissociation degrees; even in these animals K or NH_4 is more active than Na, as the difference in dissociation degree requires. These points indicate the existence of the specific actions of the cations in the stimulation phenomena of the chemoreceptor.

The stimulation of inorganic salts depends chiefly upon the nature of cations, but the anions play some part, especially in Na salts (Table 13). More precise study on the effect of the anions will be shortly reconsidered.

Among bivalent cations Ba was the only stimulant, while Ca, Mg and Sr were unable to stimulate. This and the stimulating action of HPO_4 and citrate ions (Table 13) are closely related with the observation of LOEB (1902) on frog muscle. The exception is the non-stimulation of formate in *Caudina*. In *Allolobophora*, IRWIN states that tartrate and formate of K are more stimulating than the amount of the K ions. These facts indicate the fundamental similarity of the stimulation phenomena in the different animals.

In *Allolobophora* (PARKER & METCALF '06) Ca has no antagonistic action against Na. CROZIER (1915 a) found later that, in *Rana*, the stimulating actions of Na and K are diminished by Ca. While in *Caudina*, Ca has antagonistic action against Na and NH_4 but not against the allied ions K and Li. Non-stimulation of the sea water may be due to the antagonistic actions among the component ions, for the pure salt solution has sometimes a stimulating action, in such a concentration as in ordinary sea water. OKASAKI and KOIZUMI (1927) reported that the sea water near Asamushi has the following composition (grams in 100 c.c.)

Na	K	Ca	Mg
1.1634	0.0357	0.0437	0.1373

The ratio of Na to Ca (if both expressed by molar concentration) becoms 47 : 1. The stimulation of Na is inhibited by Ca at least within the ratio of Na : Ca = 95 : 5 and 99 : 1 (Table 15). It is very probable that in a very irritable individual the stimulation of Na is antagonised by Ca in sea water. The stimulation by K ions in sea water is out of the question, for its concentration in sea water is only 0.009 M.

Acid and alkali are very efficient stimulants to almost all animals, including *Homo*. When the order of stimulation is determined by the limiting concentration or by the measurement of reaction time and the amplitude of reaction using equivalent normal solutions of various acids or alkalies, the stronger acid or alkali always excels the weaker one. This is merely due to the differences in the dissociation degrees. In *Caudina*, the order of stimulating power of acids and alkalies determined by limiting concentration is as follows (Table 16) : —

> trichloroacetic > hydrochloric > sulphuric > picric > oxalic > formic > acetic > butylic acid ;
> Sodium hydroxide > Ammonium hydroxide.

If we compare the stimulating action with the solutions containing equal H ions by the measurement of the reaction time and the amplitude of reaction, the weaker ones excel the stronger (Table 17) : —

> at pH 2.9 and 2.4   acetic > formic > hydrochloric acid ;
> pH 11.8   ammonia > Sodium hydroxide.

Recently BARÀTH and VÀNDORFY (1926) observed the same effect in human taste. The limiting concentrations of various acids for the sensory stimulation found in *Synaptula* (OLMSTED 1917), *Ascidia* (HECHT 1918 a), in *Octopus* (GIERSBERG 1926) show the same tendency. In *Ascidia*, HECHT (1918 a) found that the limiting concentrations of NaOH and NH$_4$OH to be 0.01 N and 0.015 N which are closely related with the case in *Caudina* (see Table 17). CROZIER (1916 b), HARVEY (1920) and later BARÀTH and VÀNDORFY (1926) stated that in these cases the potentially ionizable hydrogen within undissociated acid molecules plays an important part. Among these acids and alkalies, the affinity to lipoid substance and the surface activity are considerably

different.   The high stimulation power exhibited by formic and acetic acids, and also by ammonia (Table 18) is attributed to their surface activities in addition to the effect of potentially ionizable molecules. The effect of surface active substances is shown in Table 20.   CROZIER (1916 b, 1918) was the first who introduced the factor of penetration in the taste of acids, and recently TAYLOR (1927) explained the penetration of acid into living tissues.

Of the alkaloids tested, they give to human taste a bitter sensation (SKRAMILK 1926. p. 492).   In *Caudina*, only quinine, strychnine and nicotine were stimulating.   The stimulation of alkaloids has been hitherto studied by many authors in various animals (CROZIER 1915 b, OLMSTED 1917, HECHT 1918 a, CROZIER & AREY 1919, AREY and CROZIER 1919, GIERSBERG 1926, and HARNSTRÖM 1926).   In what way the reception of invertebrates for alkaloids is related with the bitter taste of human sensation, we know nothing ; however it must be noted that in any case alkaloid was stimulating in a very low concentration.

The order of stimulating effect of alcohols in *Caudina* is parallel to the well known series of narcotics (HÖBER 1926 p. 577) ; the limiting concentrations of alcohols for the human taste are 1.62 M for methyl, 0.45 M for ethyl, 0.16 M for propyl and 0.07 M for butyl alcohol respectively, according to SKRAMILK (1926, p. 190), which show the same tendency as in *Caudina*.   PARKER and STABLER (PARKER 1922, p. 171) give the following threshold values of ethyl alcohol for human sensation, 5 to 10 M for irritant, 3 M for gustatory, and 0.000125 M for olfactory sensation respectively.   In *Caudina* the threshold concentration of ethyl alcohol was about a 5 molar solution.   Hence so far as the threshold values concerned, the reception of alcohol in *Caudina* seems to belong to the common chemical reception such as the stimulation of the frog's foot by concentrated salt solutions (COLE, 1910).

The most reactive part of the body for chemical stimuli is the anterior part of the trunk, as in mechanical stimulation.   The change in the reactiveness to the chemical stimuli with the increase of body weight showed· an analogous tendency as observed in the burrowing velocity (Table 5).   In *Chiton*, AREY and CROZIER (1919) found that the reaction time of the shadow reflex becomes longer in the larger animal.   In *Caudina*, the activity became maximal in the animal of

10–20 grams without sand, and then gradually decreased, as in *Chiton*, with increase in body weight.

In general, *Caudina* is reactive to wide ranges of chemical sub-stances dissolved in water, and is so susceptible as to be compatible with human sensation. Many facts are found parallel to those found in *Homo* and other both invertebrate and vertebrate animals, which phenomena indicate the fundamental similarity of the stimulating as well as the chemo-receptive mechanism in the animal in general.

V.   HEAT.

1.   LOCAL APPLICATION OF HEAT AND COLD.

The reactions obtained from the middle trunk for the thermal stimulation are summarized as follows : —

TABLE   25.

Thermal stimulation.   Temperature of sea water 22–25°C.

Mode of stimulation	Temperature °C	Reactions	Number of animals
Local application of hot sea water	55	no reaction	
,	60	weak local contraction	
	65	the same	
	70	the same	
	75	local contraction followed by the relaxation of both circular and longitud. mus-cles; servere contractions occur on anterior and pos-terior edges of the relaxed part.	4
	80	the same	
Approaching a heated glass rod			
a. crossed to the body axis		local contraction of the circular muscles	3
b. parallel to the body axis		local contraction along the rod; longitudinal muscles are little affected	
Local application of ice piece		no reaction	

CROZIER (1915 b) observed in *Holothuria* that local application of the sea water heated to 44, 55 and 75°C. gave no reaction, except in both ends of the body, and also that no constant effect was obtained by the heated glass rod held near to the body surface; according to PEARSE (1908) *Thyone* showed no reaction to cold and heated sea water; *Synaptula* (OLMSTED 1917) and *Chiton* (AREY and CROZIER 1919) showed strong reaction both to heated and cooled sea water. *Caudina* is more receptive than *Holothuria* to thermal stimulation, but is less so than *Synaptula* and *Chiton*, especially in the response to cold substances.

It is clear that marine animals are able to react to heat and cold with definite contraction of the muscles even though the responsiveness is of less degree than for the other stimuli.

TABLE 26.

Immersion in hot or cold sea water.
Initial temperature of sea water 22–24°C.

Characteristic	Sudden immersion	Gradually heated in 17–35 minutes
Disappearance of reaction to mechanical stimulus	39°C	39°C
Beginning of relaxation	35–60	27.5–34
Recovery from, when returned to sea water.	39	38
Coagulation of the integument.	55	53–55
Number of animals	14	12

	Sudden immersion	Gradually cooled in 30–75 minutes
Disappearance of reaction to mechanical stimulus		
a) weak stimulus to the trunk	6–8°C	5–9°C
b) severe stimulus to the trunk	not at 3	1
„ „ to the tail*	10	9
Hardening of the body	--	14
Recovery from	3	1
Lowest temp. examined	3	1
Number of animals	13	7

*Shortening of the tail disappeared at the temperature indicated.

2. ALTERNATING THE TEMPERATURE OF THE MEDIUM.

When the temperature of the medium was raised suddenly or gradually, irregular constriction appeared on the body surface, and the whole body wall became relaxed and soft with no sign of muscle tonus, the body length remaining constant or slightly lengthened. Severe contraction of muscles as found by CROZIER (1915 b) in *Holothuria* at the temperature of 38–41°C was not observed in *Caudina*. OLMSTED (1917) found that in *Synaptula* the contraction occurred at 40 and at 43°C, and that it was immediately followed by relaxation. The same was found in *Caudina* when it was stimulated by local application of hot sea water (Table 25).

TAO (1927) found that both longitudinal and circular muscles of *Caudina* maintain contractility against induction shock within 5 and 40°C and that the heat rigor occurs at the following temperatures :

	beginning of shortening	marked shortening
Longitud. muscle	40	65-70
Circular muscle		65-70
Tegument		52-54

The temperature of 52–54°C, where the tegument showed marked shortening, corresponds to the coagulation temperature in my observation (53–55°C), where the outer surface became whitened. In *Caudina* the relaxation occurred at relatively low temperature (*Synaptula* at 43°C), and in spite of TAO's observation on isolated muscles, the body length remained without marked change above 39°C, and the total body became gradually hard at 53° and 55°C.

The ranges of temperature for the maintenance of the response to the mechanical stimulation are :

	upper limit	lower limit
Thyone (PEARSE 1908) .	40°C	–1.6°C
Holothuria (CROZIER 1915 b)	38–41	3
Caudina	39	1

These animals had high resistance to the low temperature, but lost all their living characteristics at the temperature of 40°C.

VI. LIGHT.

The animal was placed on the bottom of a glass jar and the reaction to photic stimuli was observed by illuminating or by making a shadow on the body surface. As a light source, a 100 watt tungsten lamp or the direct sunlight was used. Most of the heat rays were removed while the ray passed though the water layer of more than 12 cms before reaching the animal. Moreover, *Caudina* was not very responsive to radiant heat and the reaction was also slow and indefinite, therefor it must be granted that the reaction of *Caudina* to the illumination was induced only by light rays and not by unabsorbed remaining heat rays.

Caudina gave no reaction to a sudden decrease in light intensity or to a shadow made on the body surface by interrupting the light with an obstacle. It was highly reactive to sudden illumination ; in the anterior part of the body both the tentacles and the anterior end retracted ; in the posterior end the shortening of the tail took place ; and in the middle portion of the trunk, if locally stimulated, contraction occured in the part stimulated. The reaction was local if locally stimulated ; by total illumination, simultaneous reaction in both ends of the body were noticed, but in the middle part of the trunk the reaction was difficult to observe. The reaction times to sudden illumination were measured under the direct sunlight. The outside of the glass jar was covered with black paper to shut out the diffused light, and the response of the animal was observed from a small hole in the black paper, made in the side of the jar. Stimulation was produced by suddenly taking off the screen and allowing sunlight to fall on the body until the occurence of the reaction. As soon as any response was induced, the screen was inserted, and then the next exposure was made (Table 27).

In the study of the shadow reflex of *Holothuria*, CROZIER (1915 b. pp. 268, 269) found that the successive reaction times and the magnitude (retraction time) show a rhythmic character. The same is true of the successive reactions of *Caudina* to sudden illumination. Such similarity indicates the occurrence of the analogous receptor process in the receptor organs of both animals. In *Caudina* the dark reaction (HECHT, 1919 p. 162) seems to be so rapid that after 20 or 30 repeated

TABLE 27.

Successive reaction times to sudden illumination.
Illuminations (by exposure to direct sunshine) 30 seconds apart.
Experiments made in 1927.

Part observed	Anterior part of the trunk						Tip end of the tail		
No.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Date	Aug. 25.	„ 26.	„	„	Sep. 1.	„	Aug. 26.	Sep. 1.	„ 2.
Time	3 P.M.	8.30 A.M.	„	2 P.M.	1 P.M.	„	2 P.M.	1 P.M.	10 A.M.
Temp.°C	26	25	25	27			27		24
1*	1.4	1.9	1.1	2.3	1.5	2.1	1.5	2.5	2.1
2	1.3	1.9	1.1	2.4	1.6	2.1	1.5	1.9	2.1
3	1.2	1.9	1.1	2.2	1.7	2.3	1.5	1.6	2.2
4	1.3	2.5	1.7	2.1	1.6	2.2	1.5	1.9	2.2
5	1.3	2.1	1.3	2.1	1.5	1.9	1.5	2.5	2.1
6	1.1	1.9	1.4	1.8	1.5	2.3	1.8	2.1	1.9
7	1.3	1.9	1.4	1.9	1.5	1.9	1.6	2.7	2.1
8	1.2	1.9	1.4	2.1	1.7	2.1	2.1	2.8	2.1
9	1.1	2.1	1.4	2.3	1.4	1.9	2.3	2.4	1.8
10	1.5	2.2	1.1	2.3	1.6	2.1	2.2	1.9	1.9
11	1.5	1.9	1.3	2.1	1.4	2.1	1.9	2.6	1.9
12	1.9	1.9	1.3	1.6	1.9	2.6	1.9	2.3	1.7
13	2.7	1.9	1.1	2.4	1.6	2.9	1.4	1.6	1.9
14	2.2	2.1	1.3	2.1	1.8	3.0 }**	1.6	2.1	1.9
15	1.8	2.2	1.4	2.1	1.6	2.8	1.7	2.5	2.1
16	1.8	1.9	1.2	2.1	1.6	2.5	1.7	2.1	2.1
17	2.7	2.2	1.4	1.8	1.8	2.6	2.2	2.1	2.1
18	2.7	2.5	1.3	2.1	1.5	2.5	1.9	2.5	2.4
19	2.5	2.2	1.3	2.1	1.5	2.0	1.9	2.3	1.9
20	1.9	2.6	1.4	2.3	1.7	2.8	1.9	2.3	2.1
21		2.6	1.7						
22		2.4	1.5						
23		2.5	1.6						
24		2.5	1.9						
25		2.4	1.3						
26		2.5	1.8						
27		2.5	1.3						
28		2.7	1.8						
29		2.3	1.4						
30		2.6	1.6						

*1........30 indicate number of stimulus.
**In these cases the intensity of the sunshine was diminished by clouds.

stimulations the increase in the reaction times was very small.   Shadow reflex is known in the animals living on the surface of the sea bottom ; in *Holothuria* (CROZIER 1915 b), in *Thyone* (PEARSE 1908), in *Arbacia*

(UEXKÜLL 1900, HOLMES 1912), in *Chiton* (AREY & CROZIER 1919), in
Chromodoris (CROZIER & AREY 1919) and in other animals (LOEB
1918 p. 95, BUDDENBROCK 1924 p. 12). In these animals the shadow
reflexes are of importance for the protection of the body against
enemies. In *Caudina* such a reflex would be of no importance if it
existed, as the animal lives totally in the sand. As a protection for
the posterior end (the tail), the high reactiveness to mechanical stimuli
seems to be of great importance. The reaction to sudden illumination
is also found in various animals; in *Holothuria* (CROZIER 1915 b), in
Synaptula (OLMSTED 1917), in *Arbacia* (HOLMES 1912), in *Chiton*
(AREY & CROZIER 1919), in *Chromodoris* (CROZIER & AREY 1919)
and in *Mya* and *Ciona* (HECHT 1918 b. 1919). According to PEARSE
(1908), *Thyone* did not react to sudden illumination at all. Among
these animals, *Holothuria*, *Synaptula*, *Thyone* and *Arbacia* were
negatively phototropic, and *Holothuria*, *Arbacia* and *Chromodoris* col-
lected in the shade. Although the study of the phototropism of
Caudina was impossible, owing to its inactivity in locomotion out of
the sand, the response to the photic stimulus was obviously that of
avoiding the stimulus. Hence it is natural to infer that light may be
one of the factors of its life in the sand, as indicated by the relation
between light and the behavior of *Balanoglossus* (CROZIER 1917).

The holothuria have in general a specific body coloration. To
the contrary, *Caudina* has no marked body coloration; the body of
a young animal is of an opalescent pink color, which is nothing but
the color of the body fluid being seen through the thin body wall.
In the natural state, all the animals showed like coloration due to
the relaxation of all the body musculatures. In the state of tonic
contraction, the body color of the older animals became light brownish-
yellow owing to the thickening of the wall. The lack of bodily
pigmentation in *Caudina* may perhaps be due to its life in the sand,
almost shut out from light, as is seen in many cave animals.

VII. SUMMARY.

1. *Caudina* egests the ingested sand continually in intervals of
from 1 to 11 minutes; the most frequent interval is 3.5 minutes. The
time from the ingestion to the egestion is about 2 or 3 hours.

2. The animals dragged out of the sand burrow into it by the cooperative actions of the body muscles and the tentacles. The burrowing time is directly proportional to the trunk length. The burrowing velocity is not far different in any animals, and by successive trials it decreases gradually, especially in older animals. The most active ones are of the body weight 10-20 grams (without sand).

3. Locomotion on a flat surface in the air is described.

4. The entire body surface is very receptive to mechanical stimuli. The magnitude of reaction is in a decreasing order: anterior part of the trunk$>$tail$>$middle part of the trunk. Distant reaction is found in the anterior end of the trunk. On severe mechanical stimulation of one end, the opposite end remained unchanged.

5. The body muscles lose tonus on *adequate* continual mechanical stimulation. In the sand the whole body is soft and elongated; out of the sand, as the result of the muscles falling in tonic contraction, the body length becomes markedly shortened, and the relative length of the trunk and the tail reverses.

6. The natural posture in the sand is maintained by the negative geotropism of the tail, the rightening reaction (maintaining the dorsoventrality), and the positive geotropism of the trunk. The isolated tail shows strong geotropism. The tail of the imbedded animal reappears on the sand surface by its elongation.

7. The entire body surface is reactive to wide ranges of chemical substances. Stimulations are made by local application of the solutions on the body surface. *Caudina* is not reactive to the hypotonic but reactive to the highly hypertonic solutions. The stimulating order for Chlorides is: — K, $NH_4>Na>Li$; for Na-salts: — HPO_4, citrate $>Cl$, SO_4, acetate, formate. Of the four bivalent cations tested, only Ba ions are stimulating. Ca has antagonistic action against Na and NH_4, but not against K and Li; while Mg is not antagonistic against these four ions. The stimulation of acids or alkalies is chiefly accomplished by H or OH ions. With solutions containing equal H ions, the weaker acids or alkali excel the stronger ones in stimulating power. In eight alkaloids tested three are good stimulants even in a diluted solution. In alcohols, the aromatic alcohols are more effective than aliphatic; in four aliphatic alcohols, the stimulating effect is almost doubled, with one increase in CH_2. Sugars and saccharine

are not effective. The magnitude of reaction diminishes and the reaction time and its deviation increase with the dilution of the solution.

8. In chemical stimulation, the anterior part of the trunk is the most reactive. The reaction time increases only slightly by the amputation of the anterior end of the trunk. The younger animals are more active than the older. The animals of 10–20 grams (without sand) are the most reactive.

9. From the strongly stimulated spot (mechanically or chemically), sometimes peristaltic waves occur and propagate either anteriorly or, in fewer cases, posteriorly.

10. Hot sea water above 60°C and radiant heat call forth local contraction; by water heated above 75°C the local contraction was followed by the relaxation of both muscles. No reaction is obtained by the local application of ice. *Caudina* reacts to mechanical stimuli within the temperature of 1–39°C; above 40°C it loses all living characteristics.

11. The body becomes hard by rubbing the entire surface or by dropping the animal on the ground from a height of a few cms. This is also observed when the temperature of the water is lowered below 14°C (the initial temperature of sea water was 22–24°C).

12. *Caudina* shows no reaction to a sudden decrease in light intensity or to a shadow. To sudden illumination the animal responds with the contraction of the illuminated part. On repeated stimulation, the series of reaction times show a rythmic character; the increase in the reaction times is very gradual or almost neglegible.

13. The behavior of *Caudina* is quite adaptive to its environment; many facts revealed by the experiments lead us to the consideration that there exist fundamentally similar processes in the phenomena of stimulation in general.

Before leaving the subject, I wish to express my sincere thanks to Prof. HATAI, who suggested the problem, for his kind guidance and encouragement. I must also not forget the kindness shown me by Assist. Prof, KOKUBO, and Assist. KAMADA, who are members of the Asamushi Marine Biological Station.

VIII. LITERATURE CITED.

1) AREY, L. B. & CROZIER, W. J. 1919. The sensory responses of *Chiton*. J. Exp. Zoöl., Vol. 29, pp. 157–256.

2) BARÀTH, E. u. VÀNDORFY. 1926. Experimentelle Untersuchungen über die physikalisch-chemischen Grundlagen der Geschmacksempfindung. Bioch. Z., Bd. 176, p. 473.

3) BUDDENBROCK, W. v. 1912. Über die Funktion der Statozysten im Sand grabender Meerestiere (*Arenicola* u. *Synapta*). Biol. Zentralb., Bd. 32, p. 564, and in Zool. Jahrb. Abt. Allg. Zool. u. Physiol., Bd. 33, p. 441, 1913.

4) BUDDENBROCK, W. v. 1924. Grundriss der vergleichenden Physiologie. I. Teil: Sinnesorgane und Nervensystem. Berlin. p. 12.

5) COLES, L. W. 1910. Reactions of Frogs to Chlorides of Ammonium, Potassium, Sodium and Lithium J. Comp. Neurol. Psychol., Vol. 20, pp. 601–614.

6) CROZIER, W. J. 1915a. Ionic Antagonism in sensory Stimulation. Amer. J. Physiol., Vol. 39, pp. 297–302.

7) CROZIER, W. J. 1915b. The sensory reactions of *Holothuria surinamensis* LUDWIG. Zool. Jahrb. Abt. Allg. Zool. u. Physiol., Bd. 35, pp. 233–292.

8) CROZIER, W. J. 1916a. The rythmic pulsation of the cloaca of Holothurians. J. Exp. Zool., Vol. 20, pp. 297–353.

9) CROZIER, W. J. 1916b. The Taste of Acids. J. Comp. Neurol., Vol. 26, pp. 453–462.

10) CROZIER, W. J. 1917. The photic Sensitivity of *Balanoglossus*. J. Exp. Zoöl., Vol. 24, pp. 211–216.

11) CROZIER, W. J. 1918. Sensory Activation by Acids. Amer. J. Physiol., Vol. 45, pp. 323–341.

12) CROZIER, W. J. & AREY, L. B. 1919. Sensory reactions of *Chromodoris zebra*. J. Exp. Zoöl., Vol. 29, pp. 261–308.

13) GEROULD, J. H. 1896. The anatomy and Histology of *Caudina arenata* GOULD. Bull. Museum Comp. Zool. Harvard College, Vol. 29.

14) GIERSBERG, H. 1926. Über den chemischen Sinn von *Octopus vulgaris*. Z. Vergl. Physiol., Bd. 3, p. 827.

15) GRUTZNER, P. 1894. Über die chemische Reizung sensibler Nerven. Pflüg. Arch., Bd. 58, p. 69.

16) HARNSTROM, B. 1926. Einige Experimente und Reflexionen über Geruch, Geschmack und allgemeinen chemischen Sinn. Z. verg. Physiol., Bd. 4, p. 528.

17) HARVEY, R. B. 1920. The Relation between the Total Acidity, the Concentration of the Hydrogen Ion, and the Taste of Acid Solutions. J. Amer. Chem. Soc., Vol. 42, pp. 712 714.

18) HECHT, S. 1918a. The Physiology of *Ascidia atra* LESUEUR. II. Sensory physiology. J. Exp. Zoöl., Vol. 25, pp. 261–297.

19) HECHT, S. 1918b. The photic Sensitivity of *Ciona intestinalis*. J. Gen. Physiol., Vol. 1, pp. 147–166.

20) HECHT, S. 1919. Sensory equilibrium and dark adaptation in *Mya arenaria*. J. Gen. Physiol., Vol. 1, pp. 657–685.

21) HÖBER, R. 1926. Physikalische Chemie der Zelle und der Gewebe. Leipzig. p. 577.

22) HOLMES, S. J. 1912. Phototaxis in the sea-urchin *Arbacia punctulata*. J. Anim. Behavior., Vol. 2, p. 126.

23) IRWIN, 1918. The nature of sensory Stimulation by Salts. Amer. J. Physiol., Vol. 47, p. 276.

24) JORDAN, H. 1914. Über reflexarme Tiere. IV. Holothurien. Zool. Jahrb. Abt. Allg. Zool. u. Physiol., Bd. 34, p. 422.

25) KAWAMOTO, N. 1927. The anatomy of *Caudina chilensis* (J. MULLER). Science Reports, Tôhoku Imp. Univ. (Biology), Vol. 3, pp. 239-262.

26) LEWIS, 1923. System of Physical Chemistry. Vol. I.

27) LOEB, J. 1902. Ist die erregende und hemmende Wirkung der Ionen eine Funktion ihrer elektrischen Ladung? Pflüg. Arch., Bd. 91.

28) LOEB, J. 1918. Forced Movements, Tropisms and Animal Conduct. Philadelphia and London. p. 95.

29) MICHAELIS, L. 1922. Die Wasserstoffionen-konzentrationen. Berlin. p. 21.

30) OKAZAKI, K. & KOIZUMI, T. 1927. Über die Leibeshöhlen-flüssigkeit von Holothurien, *Caudina chilensis* (J. MULLER). Science Reports, Tôhoku Imp. Univ. (Biology), Vol. 2, pp. 141-142.

31) OLMSTED, J. M. 1917. The comparative Physiology of *Synaptula hydroformis* (LESUEUR). J. Exp. Zoöl., Vol. 24, pp. 333-376.

32) PARKER, G. H. 1922. Smell, Taste, and Allied Senses in the Vertebrates. Philadelphia and London. pp. 138-139, 171.

33) PARKER, G. H. & METCALF, C. R. 1908. The Reactions of Earthworms to Salts. Amer. J. Physiol., Vol. 17, pp. 55-69.

34) PEARSE, A. S. 1908. Observations on the Behavior of *Thyone briareus* (LESUEUR). Biol. Bull., Vol. 15, pp. 259-287.

35) SKRAMILK, E. v. 1926. Handbuch der Physiologie der Niederen Sinne. I. Bd. Leipzig. pp. 490-492.

36) TAYLOR, N. W. 1927. Acid Penetration into living Tissues. J. Gen. Physiol., Vol. 11.

37) TAO, L. 1927. Physiological Characteristics of *Caudina* Muscle. Science Reports, Tôhoku Imp. Univ. (Biology), Vol. 2, pp. 265-291.

38) UEXKÜLL, J. v. 1921. Umwelt und Innenwelt der Tiere. Berlin. p. 54.

39) UEXKULL, J. v. 1926. Die Spermuskulatur der Holothurien. Pflüg. Arch., Bd. 212, p. 1.

40) WOLF, E. 1925. Physiologischen Untersuchungen über das Umdrehen der Seesterne und Schlangensterne. Z. verg. Physiol., Bd. 3, p. 209.

41) YAMANOUCHI, T. 1926. Some Preliminary Notes on the Behavior of Holothurian, *Caudina chilensis* (J. MÜLLER). Science Reports, Tôhoku Imp. Univ. (Biology), Vol. 2, pp. 85-91.

42) YAMANOUCHI, T. 1929. Statistical Study on *Caudina chilensis* (J. MULLER). Ibid. Vol. 4, No. 2, in press.

Study of *Euryale ferox* SALISB.
III. On the Form and Structure of Juvenile Leaves.

By

Yônosuke Okada.

(With Plates X–XI.)

In my previous paper[1], I reported on the developmental course of the leaves of the seedling of *Euryale* and gave a brief description of the external appearance of the juvenile leaves. (Lately I have learned that the fact was first noticed in this country by Dr. Makino.[2]). Since that time I have been engaged in the study of the plant and in particular with respect to the problem of the delayed germination of the seeds, of which phenomenon I hope to report in the future. Furthermore, in the late spring of this year, I had the opportunity to make some further observations on the development of the leaves from the seedling, and I have accordingly undertaken to give here a second report on the matter to substantiate the previous paper.

The study was performed for the most part with the seeds collected at Zyûnityôgata[3], in the autumn of 1926 and 1927. The seeds were thenceforth kept submerged[4]. A number of these seeds germinated in the late spring of this year, and the observation in the present paper was carried on with them. A few of the samples from other sources, as Hukusimagata, Ohnuma, Tatenuma and Oguranoike, were also subjected to the study, and the observed data on them also are annexed at the close of the paper.

[1] Okada, Y. 1925. On the germination of *Euryale ferox* Salisb. Bot. Mag. Tokyo, Vol. 39, pp. 133–141.

[2] Makino, T. 1891–97. Botanical notes from the author's private cabinet. p. 2. (Text in Jap.)

[3] For this locality and others, see the previous paper: Okada Y 1928. Study of *Euryale ferox* Salisb. I., Sci. Rep., Tôhoku Imp. Univ., Sendai, Ser 4, Vol. 3, p. 272.

[4] Detailed description on the condition of storage will be given in the future in connection with the problem of the delayed germination. Hence the abridgement here.

So much for the origin of the material. Let us now proceed to the discussion of the work proper.

The types of leaves developing consecutively from the'¡ seed of
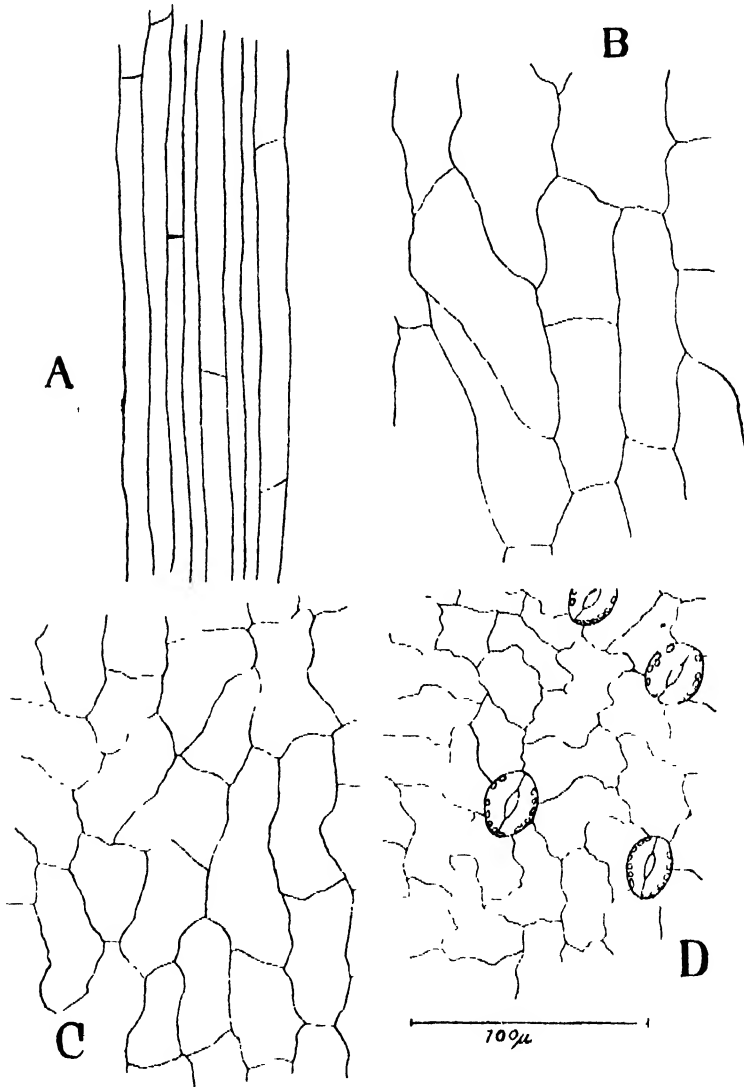
Text-fig. A — D. Epidermal cells of the juvenile leaves. A. first leaf; B. second leaf; C. third leaf; D. fourth leaf.

Euryale follow, as a general rule, the normal order already described in the previous report. This order may therefore be regarded as the regular type for the Zyûnityôgata-form. The consecutive leaves appear in this case in the following order; first, subulate, submersed; second, narrow hastate, submersed; third, broad hastate, submersed; fourth, fissi-cordate, floating (Pl. X, fig. 1).

After the fourth leaf, the transformation proceeds rather gradually in contrast to the transformations seen in the preceding leaves, and further study beyond the fourth leaf was accordingly not undertaken.

The distinction among the first four leaves is very remarkable not only in the external form of the leaf blade but also in the internal structure. Of these four leaves, the second and the third assume somewhat common characters, while the first and the fourth exhibit a distinct contrast. In the following, the remarkable points of difference among these leaves are tabulated.

Thus we can see that the distinctions are remarkable in diverse

Ist.	IInd. & IIIrd.	IVth.
1. Submersed.	Submersed.	Floating.
2. Length of leaf predeterminate.	Length of petiole predeterminate.	Length of petiole very plastic according to the water depth.
3. Lamina undifferentiated from petiole.	Lamina differentiated (hastate)	Lamina differentiated (fissicordate)
4. Epidermal cell elongated.	Epidermal cell of the upper surface tends to be isodiametrical, and larger than that of the fourth leaf.	Epidermal cell of the upper surface isodiametrical.
5. Stoma absent.	Stoma usually absent.	Stomata abundant.
6.	Lamina thin, translucent and flaccid.	Lamina tough leathery, the upper surface relatively non-wettable.
7. Palisade tissue undifferentiated.	Palisade tissue undifferentiated.	Palisade tissue well differentiated.
8.		Violet coloration of the lower surface accentuated.
9.		Tencile strength of the petiole developed.

points. We will treat of these characters in the following paragraphs.

1) Submersed or floating. It is self-evident that this distinction can be clearly discerned only when a sufficient depth of water is afforded. To take an extreme case, if we allow to the seedling water only one or two cm. deep, all the leaves must grow erect beyond the water surface and the distinction naturally disappears. Except in such abnormal cases, the first three leaves remain submersed while the fourth leaf comes to the water surface where the blade expands to its full size. The cause of this distinction we may find in the next section.

2) Extract from the record of the measurement of the length of the petiole (in the case of the first leaf, the total length of the leaf was measured, as the differentiation of the lamina from the petiole does not take place), as well as the water depth applied is given in the table below.

Water depth	Length of the petiole (in cm.)				
	Ist.	IInd.	IIIrd.	IVth.	Vth.
6	8	7	9.5	35	31
6	6	8.3	8.3	33	35
7	--	10	16	36	—
7	–	11	11	37	--
8	10	10.5	12.5	34	28
8	11	9.1	12	34	--
16	6	5.0	10	33	—
16	8	5.8	6.8	28	—
29	7.5	5	6.2	39	41
29	10	6	7.8	39	43
80	11	8	10	94	—

Of the values recorded in the table, some cases may be noticed in which the length of the leaves to be submersed nevertheless exceeds the depth of the water, a seemingly paradoxical phenomenon. In reality, those leaves grow upward not vertically but in an oblique direction, so that the lamina may remain always under water.

We can learn from this table that the length of the first three leaves is rather uniform regardless of the depth of the water. The range of variation is narrow in comparison with the change in water depth. The length varies for the first leaf, from 6 to 11 cm., for the second, from 5 to 11 cm., and for the third from 6.2 to 12.5 cm.,

while the water depth changes from 6 to 80 cm. Quite different is the case with the fourth leaf, where, although the length of the petiole is not very variable (28–34 cm.) as far as a water depth of 16 cm., it is able to elongate as long as 94 cm. when cultivated in 80 cm. deep water.

These facts show that the length of the petiole is predeterminate in the first three leaves, while it is quite plastic in the fourth. The latter petiole elongates always more or less longer than the water depth applied and allows the leaf blade to float on the water surface.

3) As for the form of the leaf blade, no additional remark is necessitated beyond the description in the previous report. In Pl. X, fig. 1 are shown the general features of the consecutive leaves. Of these types of leaf form, the subulate appearance of the first leaf is of quite common occurrence among *Nymphaeaceae*[1].

4) The epidermal cells of the first leaf are much elongated all over the surface and are arranged in parallel with the axis of the leaf itself (text-fig. A). The same cells of the next two leaf blades tend to be isodiametrical and gradually reduced in size (Text-fig. B, C). The fourth leaf blade has the smallest epidermal cells of the four, and no shade of parallel arrangement can be discerned. Moreover, the sinuous appearance[2] of the suture line between the neighboring cells is quite accentuated (Text-fig. D).

5) Since the astomaticism of some aquatic plants was first noticed by BRONGNIART[3], the difference in the distribution of stomata between the aerial and submerged part of the plant body was studied by

[1] For *Nymphaea spp.*, GOEBEL, K. 1893. Pflanzenbiol. Schilderungen, 2 Teil, 2 Lief., p. 303; TRICKER, WM. 1897. The water garden, New York (cit. CONARD); CONARD, H. S. 1905. The waterlilies, p. 109; ARBER, A. 1920. Water plant, a study of aquatic angiosperms, pp. 35–36: for *Castalia alba*, ARBER, A., 1920 l. c, p. 28: for *Victoria regia*, GWYNNE-VAUGHAN, D. T., 1897. On some points in the morphology and anatomy of the *Nymphaeaceae*. Trans. Linn. Soc., London, Ser 2, Vol. 5. Pt. 7, pp. 287–299 (cit. ARBER).

[2] Analogous case of contrast with respect to the sinuosity is noticed by ARBER (1920. l. c., p. 163) between the floating and submersed leaves of *Callitriche verna*.

[3] BRONGNIART, A. 1834. Nouvelles recherches sur la structure de l'épiderme. Ann. d. Sci. nat., 2 Sér. 1. pp. 65–71. (cit. GOEBEL).

many authors[1]. Thanks to the effort of these workers, many inter-
esting data were found concerning the problem. In some cases, the
presence or absence of the stomata is predetermined strictly by heredity,
and the surrounding medium affects in no way their distribution. For
example, *Isoetes Malinverniana*, *Is. Boryana*, *Is. tenuissima* and *Is.
Perralderiana* are reported by GOEBEL to have stomata regardless of
their aquatic life, while, on the contrary, *Isoetes lacustris* and *echino-
spora* never produce stoma even when grown as land forms[2]. On the
other hand, in some plastic forms as *Hippuris*, *Proserpinaca*, *Myrio-
phyllum*, *Stratiotes* and *Utricularia*, the presence or absence of the
stomata on the leaf is correlated with its emergence or submergence[3].
Between these extreme cases, many transitional degrees[4] of correlation
of stomata formation and water are reported. For instance, some
plants are reported to have more stomata on the aerial than on the
submersed part (or more stomata in the land form than water form),
and still others are noted to produce more stomata in shallow water
than in deep water. Thus, although it seems inappropriate to establish
a uniformity among these different cases, we can still conceive in
many cases the tendency that the contact with free air favors the
production of the stomata.

As for the same problem with the *Nymphaeaceae*, the presence of
the stomata on the aerial or the floating leaves (always on the upper
surface only) is generally recognized. With the submersed leaves,

---

[1] GOEBEL, K. 1893. l. c., pp. 237-245; PORSCH, O. 1903. Zur Kenntnis des Spalt-
öffnungsapparates submerser Pflanzenteile. Sitzb. Akad. Wien, Mathem-naturw.
Klasse, Vol. 112, Abt. 1, pp. 97 138; SOLEREDER, H. 1913. Systematisch-anatom-
ische Untersuchung des Blattes der *Hydrocharitaceen*. Beih. Bot. Zentrbl., Vol.
30, Abt. 1, pp. 24-104; ARBER, A. 1920. l. c.

[2] GOEBEL, K. 1893. l. c., p. 241.

[3] GOEBEL, K. 1893. l. c., pp. 240-244; PORSCH, O. 1903. l. c., p. 106; COWLES, H.
C. 1911. Ecology, pp. 561-2. (in COULTER, BARNES and COWLES: Textbook of
Botany). As for *Hippuris*, however, somewhat contradictory report is given
that the stomata are formed, during the vigorously vegetating season, on the
leaves of this plant when the leaf anlage is still submerged. c. f. CONSTANTIN, J.
1886. Étude sur les feuilles des plantes aquatiques. Ann. d. Sci. nat., 7 Sér.
Bot., T. 3, pp. 94-162. (cit. ARBER).

[4] GOEBEL gives a detailed description of various cases, c. f. GOEBEL, K. 1893. l. c.,
pp. 240-244.

however, astomaticism is reported by GOEBEL[1] in *Nuphar* and *Cabomba*, and by BRAND[2] in *Nymphaea alba*, while positive cases are observed by CONARD[3] in *Nymphaea spp.*, by ARCANGELI[4] in *N. alba*, by WÄCHTER[5] in *N. spp.*, and by GOEBEL[6] in *N. coerulea*. It seems that the presence of stomata on the submersed leaves of *Nymphaea* is at least not absolutely excluded.

So much for the problem in general. Now let us consider the same case with *Euryale*. As already stated above, the first three leaves from the seedling of *Euryale* are submersed and the fourth leaf becomes firstly the floating type. The distribution of stomata on these leaves is described below : —

The first leaf is possessed of no stoma at all (number of observed samples being 15). Nor has the second leaf any as a general rule. Only one case of exception was observed out of 17 samples. This example was, however, one of an individual cultivated in shallow water (about 2 cm. deep) and the leaf had probably by chance been exposed to the free air. Whether there is any relation between the formation of the stomata and the exposure to the air was not experimentally determined in my study. The fact is that the number of stomata in the above example was very small, only some 30 being found all over the upper surface.

As for the third leaf, in 5 samples out of 14, the presence of stomata was positively demonstrated. Of these five, four cases were observed with individuals in shallow water (2-6 cm. deep), and the chance of exposure to the air must be suspected of them. Even in these positive examples, the number of stomata was by far the smaller as compared with that on the fourth leaf. The actual numbers of

[1] GOEBEL, K. 1893, l. c., p. 242.

[2] BRAND, F. 1894. Über die drei Blattarten unserer *Nymphaeaceae*. Bot. Zentrbl., Vol. 57, pp. 168–171.

[3] CONARD, H. S. 1905. The Waterlilies, p. 77.

[4] ARCANGELI, G. 1890. Sulle foglie delle piante acquatiche e specialmente sopra quelle della *Nymphaea* e del *Nuphar*. Nuovo Giorn. Bot It., Vol 22, pp. 441–446. (cit. CONARD).

[5] WÄCHTER, W. 1897. Beiträge zur Kenntniss einiger Wasserpflanzen. III. Über die Abhängigkeit der Heterophyllie einiger *Nymphaea* arten von äusseren Einflussen. Flora, Vol. 84, pp. 343-348.

[6] GOEBEL, K. 1893. l. c., p. 242.

stomata on the third leaf were 3, 11, 2, and 22 per sq. mm. with the above four examples. With leaves absolutely kept from contact with the air, the stoma was generally found to be absent. This is, however, not absolutely always the case. In one case out of seven under such a condition, the presence of stomata was positively proved, though the actual number was exceedingly small (about 20 in total all over the surface).

The fourth leaf never fails to produce the stomata. There is allowed no exception (number of observed samples being 20). Moreover, they are very abundantly produced. Six examples of fully grown leaves were subjected to the actual counting. The number of stomata on these six were 221, 199, 220, 226, 216, and 218 per sq. mm. of the leaf surface each (These values concern the more central region of the leaf blade. The peripheral region yields somewhat lower values. With the same six samples as above, the numbers of stomata in the periphery of the lamina were 168, 183, 175, 196, 177, and 175. All these numerals represent the averages of four measurements each.).

The dimension of the stoma is fairly uniform all over the surface except around the so-called astomatic region[1] near the insertion of the petiole, where specially large ones are arranged. The major axis of the stoma is 29.95 μ (average of 20 measurements) long in the normal one and 47.1 μ (average of 20 measurements) long in the larger one, so that the latter is some 50% as larger as the former (The numerals concerning the density of stomata on the leaf surface given above relate to the area of the normal sized stomata.).

The formation of stomata on the fourth leaf is commenced far before the appearance of the lamina on the water surface. Therefore, we can in this case at least prove positively the independence of the stoma formation from the contact with the free air.

N. B. All the remarks on stomata concern solely the upper surface of the lamina. No stoma is observed on the lower surface at all.

6) The upper surface of the fourth leaf is decidedly more coriaceous than the others and cannot be easily moistened. This distinction is probably due to the better development of the cuticle in the fourth leaf than the other three.

[1] CONARD, H. S. 1905. l. c., p. 65.

7) The fourth leaf is possessed of a markedly differentiated palisade layer on the upper side of the lamina, consisting of two or three layers of cells. The third and second have no such layers. Only the subepidermal layer of the upper side of these leaves may be distinguished with the presence of somewhat abundant chloroplasts. The first leaf has no differentiated lamina and still less of a palisade tissue[1].

8) The red coloration (probably the anthocyanin pigment) is found in any of the four in general. At any rate, the epidermal cells on the lower surface of the fourth leaf are decidedly rich in the same and assume a violet color.

9) The petiole of the fourth leaf is quite resistant against traction, while the others submit themselves most easily to the stress.

All these points show that the juvenile leaf of *Euryale* suffers a fundamental transformation during its course of development. Another fact to be noticed is that this order of transformation is of quite a fixed character. It is followed quite faithfully so far as the Zyûnityôgata-form of *Euryale* is concerned, so that we may for brevity denominate this type of developmental course as the Z-type. It was, however, sometimes observed that the last of the submersed leaves was omitted from the course of development, that is to say, the third leaf of the seedling appears as a floating leaf, with well developed stomata and palisade tissue and having its petiole decidedly elongated (The number of stomata were counted with three individuals of this type, the results being 237, 210 and 167 per sq. mm. for the central part of the lamina and 242, 192, and 129 per sq. mm. for the periphery.). Out of 74 examples studied this spring, 68 (92%) belong to the normal Z-type, only the remaining 6 representing exceptions.

As for the individuals from other localities, of which the general tendency is found to coincide with the Zyûnityôgata-form, brief remarks will be given below.

15 examples of Hukusimagata-plants were studied. All of them had their third leaves floating on the water surface. No exception was observed, and this type of development seems therefore characteristic

[1] The difference in the development of the palisade tissue between submersed and aerial leaves is often observed. GOEBEL, 1893. l. c., p. 246; WÄCHTER, W. 1897. l. c., p. 343; SOLEREDER, H. 1913. l. c., p. 34.

of them.   We may denominate it as the H-type (Pl. XI).

Of the plants from Ohnuma and Tatenuma, 12 and 8 examples were examined respectively, all of which were proved to assume the Z-type.

Lastly, 4 examples of Oguranoike-plants gave 2 of Z-type and 2 of H-type.   Owing to the shortage in the material, no definite result could be attained in this last case.

In conclusion, the writer wishes to express his indebtedness to the Department of Education for the subsidy to promote the natural sciences, with which the cost of the present study was partly defrayed.

EXPLANATION OF THE PLATES.

PLATE X.

Fig. 1.   Seedling of the Zyûnityôgata-plant. Z-type, the third leaf is of submersed type (The numerals indicate the order of the consecutive leaves in the developmental course.).   Water depth 16 cm.

Fig. 2.   The same. H-type, the third leaf is of floating type, having its petiole much elongated.   Water depth 16 cm.

PLATE XI.

Fig. 1.   Seedling of the Hukusimagata-plant. H-type, the third leaf is of floating type.   Water depth 16 cm.

Fig. 2.   The same. The third leaf is of floating type.   The distinction between the petioles of 2nd and 3rd leaves are quite in contrast.   The first leaf has degenerated away in this example.

ERRATUM.

In my first report in this periodical, Vol. 3, no. 3, fasc. 1, p. 272, line 3, for **Mao** read **Mo-o**.

Fig. 1.

Fig. 2.

Y. OKADA: *Euryale ferox* SALISB.
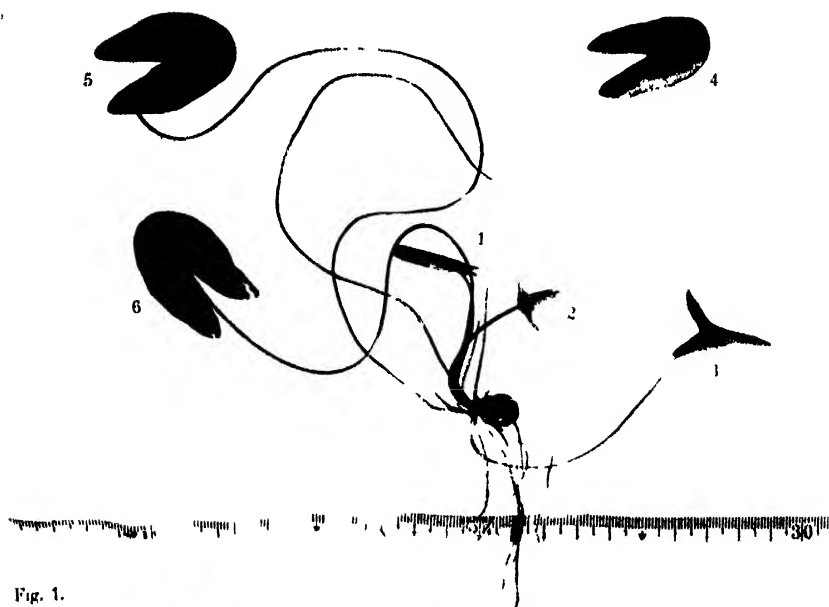
Fig. 2.

Fig. 1
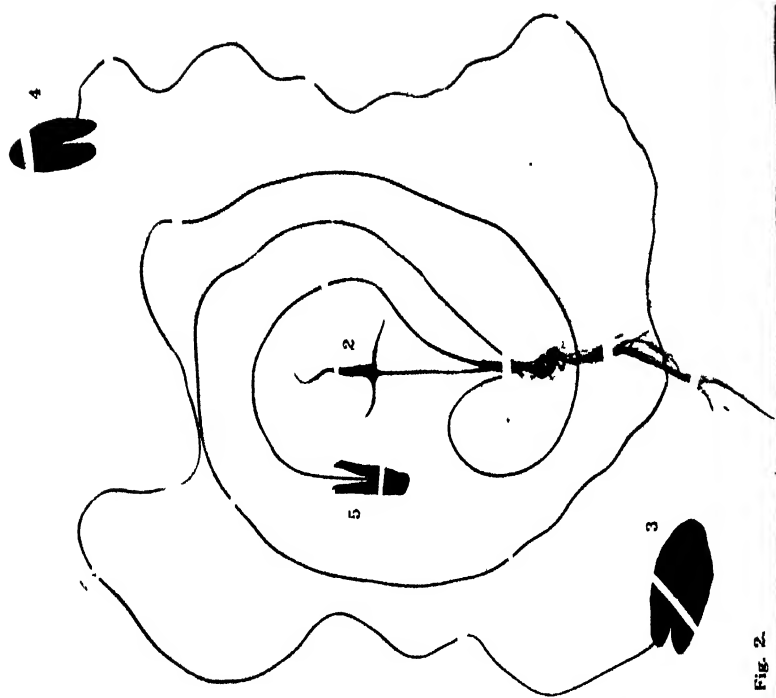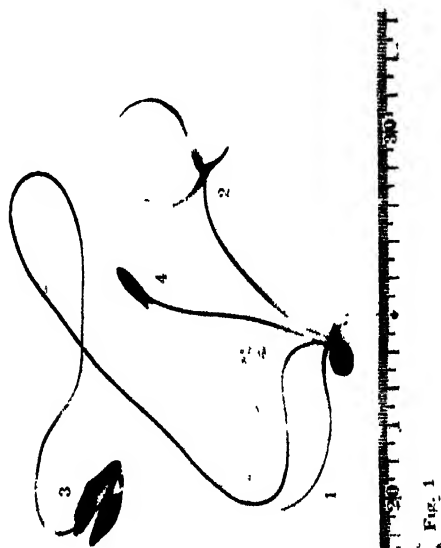
Y. OKADA : *Euryale ferox* SALISB.

Notes on the Germination of the Spores of some Pteridophytes with Special Regard to their Viability.

By

YÔNOSUKE OKADA.

INTRODUCTION.

So far as the morphological point of view is concerned, the fern spore is an entirely different body from the seed of a phanerogam. As for the biological significance of the two, however, they have much in common, since they both have to fulfill their function as a disseminule; hence their frequent juxtaposition in noted textbooks on plant ecology (CLEMENTS, 1907, pp. 241-2; COWLES, 1911, p. 805; TANSLEY, 1923, p. 18.). They resemble each other, indeed, in many points, e. g. in resistance against extreme conditions, retaining of viability over a long period, recovery of activity or germination in case of favorable conditions, and so on. We know that seeds have much to do with human life; they are of the utmost significance not only in agri-, horti-, or sylviculture but also in industry. It is therefore easily comprehensible why hundreds of workers have devoted themselves to the study of the seed, from divers directions. Thus the question of the viability of the seed has been treated by many authors, and results of elaborate studies are to be found, for instance, in the work by GUPPY (1912). The question of the conservation of viability has also been much discussed.

On the other hand, studies on the physiology of fern or moss spores, if not actually rare, are not at all to be compared in number with those on seeds. The question of the longevity of spores seems in particular not to be favored, and we can find only occasional mention of this point in literature[1]. Much less treated even is the

---

[1] In the paper by HARTT (1925, pp. 430-431), concerning literatures are to be found and therefore no revision may be necessary here.

decline of viability, which phenomenon is the general rule for fern spores. They are destined to exhibit the highest germination capability at the time of being shed from the mother sporangia, and later, as the days go on, they are sure to suffer more or less rapidly from the decrement in vitality. In extremely rare cases, attainment of full germination capacity after a lapse of time following the liberation from the sporangia is observed ; for instance, LIFE (1907) reports of dormancy in the spores of *Alsophila*, and in the textbook of Ecology by COWLES (1911, p. 932) it is described that the spores of *Lycopodium* display a dormant period. Now, considering our meager knowledge of this subject, it may be worth while to pursue the problem further. In particular, such question as the influence of the condition of storage on longevity may not be of minor significance from the viewpoint of general biology.

The fern spores, being of a unicellular structure, have excessively minute size and offer no convenience for manipulation in the procedure of experiment. But, on the other hand, the same circumstances allow us to study the effect of external factors directly influencing them. Moreover, the fact that we are able to experiment with a sufficiently large number of individuals in a comparatively small space is quite a favorable condition for finding out the average features under controlled millieu. For these reasons the writer has undertaken the study of the present problem.

An experiment was first made to obtain the general features concerning the relation between the environmental factors and germination of the spore. As the factors of importance influencing the germination, were considered to be temperature, illumination, culture medium, and oxygen supply, each of their effects were studied. Secondly, making use of the knowledge thus obtained, it was endeavoured to obtain a combination of the most favorable conditions for germination, and under such a standardized condition, the studies on the germination capability were carried on with the spore materials kept in various storage conditions. The decrement in the germination capability due to age was traced until the per cent of germination was reduced to nil, which point was assigned to be the limit of the life. Thus the difference in the span of life among different species and by different methods of storage was established. Finally, some

of the properties of spores were brought under experiment, to study if there existed any correlation between them and the viability of the spore.

The present study, which was carried on with these problems just described in view, was first undertaken, when I was in the Botanical Institute, Tokyo Imperial University, under the guidance of Prof. Dr. MIYOSHI, to whom I wish to express gratitude for his valuable suggestions. After my removal to Sendai, the study was continued there. My thanks are due to the members of the Institute, with whose encouragement and aid the study has proceeded.

MATERIALS OF THE STUDY.

The materials of the present study are the spores of the six species

Species	Date	Locality	Lot
I. *Equisetum arvense* L. (スギナ)	25/IV 1925	Tunagi near Sendai.	No. 5
,,	10/IV 1926	,,	No. 6 a
,,	18/IV 1926	,,	No. 6 b
,,	25/IV 1926	,,	No. 6 c
,,	2/V 1926	,,	No. 6 d
,,	17/IV 1927	,,	No. 7
II. *Osmunda japonica* THUNB. (ゼンマイ)	7/VI 1925	Mt. Idumigatake near Sendai.	No. 5
,,	2/V 1926	Kame-ga-mori near Sendai.	No. 6 a
,,	16/V 1926	Gongen-no-mori near Sendai.	No. 6 b
,,	18/V 1927	,,	No. 7
III. *Osmunda cinnamomea* L. (ヤマドリゼンマイ)	7/VI 1925	Mt. Idumigatake near Sendai.	No. 5
,,	28/V 1926		No. 6
,,	22/V 1927	,,	No. 7
IV. *Dryopteris viridescens* O. KUNTZE. (リョウメンシダ)	15/III 1925	Dusi, Kanagawa Prefecture.	No. 5
,,	28/III 1926	Ohmori near Sendai.	No. 6
,,	9/III 1928	Dusi, Kanagawa Prefecture.	No. 8
V. *Matteuccia struthiopteris* (L.) TODARO. (クサソテツ)	4/III 1925	Bot. Gardens, Koisikawa, Tokyo.	No. 5
VI. *Woodwardia orientalis* SWARTZ. (コモチシダ)	15/III 1925	Dusi, Kanagawa Prefecture.	No. 5
,,	9/III 1928	,,	No. 8

of pteridophytes, of which the localities and dates of collection are
tabulated on the preceding page.

Of these six species, I, II, III and V possess specialized sporangia-
bearing fronds. In their natural habitat in the fields were collected
those fronds of which the sporangia were quite mature and the spores
ready to be shed off even with a slight touch of the hand. Those
sporophylls were packed in small paraffin paper sacks and brought to
the laboratory, where they were spread over paraffin paper sheets on
a bench for one or two days. In the meantime the spores were sure
to be shed freely from the fully ripe sporangia, and only these freely
liberated spores were collected for the purpose of experimental study,
all the rest remaining in the sporangia being discarded.

The collected mass of the spores was naturally not quite pure,
but was mixed with such impurities as fragments of sporangia, trichoms
etc. Sifting several times through a 0.04 mm. mesh and then blowing
off the trifling impurities which still remained with a gentle air current
from a blast pump, were quite effective as a method of purification,
even though not a small portion of the available materials were also
lost in the process of the treatment. As we will see in a later chapter,
since the viability of spores decreases rather rapidly under ordinary
laboratory conditions, the collected materials were presently put into
an ice chamber, except in experiments for special purposes, either in
dry condition or not, according to the specific keeping properties of
the species.

The other two species, viz., *Woodwardia* and *Dryopteris* have no
specialized sporophyll, so that we had to collect a large quantity of
material to start with. The same process of purification was applied
as in the case of the other four, and equally satisfactory results were
attained, except that quite a large quantity of the original raw
materials was necessary.

METHODS.

In the present study, miniature PETRI dishes (4 cm. wide and 1 cm.
deep) were employed invariably as germinator vessels for the spore.
In these small dishes was put the culture medium, which consists of
0.1 % KNOP's solution added with 1% agar-agar. For the most part

of study, this medium was clinged to, except in the experiment concerning the effect of the substratum and in such cases as are provided with special remarks. Before sowing the spores, the germinator vessels with culture media in were autoclaved, though the procedure was not valid enough to suppress the microbial infection perfectly, as we are not capable of obtaining the spore material in absolutely germ free condition. An absolutely aseptic culture in the physiological study of the fern spore seems not to have been hitherto established, mainly due to the difficulties in sterilizing the spore material. (On this point, care of KREBS, 1916, p. 13; STEPHAN, 1928, p. 386.). Application of Potassium-permanganate to sporangia (LANG, 1898; TWISS, 1910, p. 170; ROGERS, 1923, p. 76.) was not applicable as I resorted solely to those spores which are shed freely from the sporangia. Yet, the autoclaved substratum yielded much better results and the procedure was maintained throughout the study. Though the microbial contamination was not absolutely excluded, yet the substratum suffered only a little from them mainly due to the lack of available organic matter in the medium, especially when the spores are afresh and of vigour. After the spores are reduced of their vitality in later days, heavy infection sometimes falled in and we were compelled to discard the culture in such cases.

The spores were taken out from the storage receptacle by means of sterilized platinum needle, and with a light shock of hand applied to the needle we can obtain quite a uniform distribution of spores over the substratum. The course of germination was studied under microscope from time to time, usually with the combination of ZEISS aa × 4, or sometimes with AA × 4 when detailed observation was necessary. The appearance of the first cell division of the spore was adopted as the criterion for the germination capability[1]. In general, the germination of the fern spores studied was found to be carried on quite rapidly, so long as they were fresh, and those which had initiated the first division did not fail to produce the prothallium in due time, provided under proper conditions. Old spores, however,

---

[1] Other criteria, however, may be adopted as well. Thus, for instance, LAAGE (1907, p. 79) accounted those spores with the exine ruptured as capable of germination, and STEPHAN (1928, p. 387) estimated the first definite appearance of the rhizoid and the green cell also as such.

owing to the decrease of vitality during the storage, are sometimes devoid of sufficient germination energy to perform the normal development and remain inactive after the first division has been completed. Since the further development was not specially regarded in our study, all those indicating the first cell division were reckoned as equally vital, regardless of the fate of the prothallia. Counting was made with two or three hundred spores and the number of the germinated spores was expressed in per cent (so-called " Zählmethode " by LAAGE, 1907, p. 110).

These are the general features of the methods in the present work. Specified procedure will be given later in each separate section.

I. ON THE EXTERNAL CONDITIONS.

CULTURE MEDIUM.

In studying the development of fern prothallia from spores, so far as it concerns only the morphological point of view, any substance available, such as bricks, garden turf, etc. may be employed as a medium on which to sow the spores. In the present study, however, as the per cent germination is taken into account for judging the viability of the spore, counting under the microscope is imperative, and accordingly a transparent substratum is by far preferable. For this purpose, two kinds of culture media were compared, viz., a liquid nutritive medium and the same stabilized with agar-agar. To determine which of the two, the liquid or solid medium, was more favorable for the germination of the spore, the following experiments were undertaken. As it was learned by preliminary study that KNOP's nutritive solution of 0.1% or thereabout is quite favorable for the germination of the spore, this liquid medium and the same medium solidified with 1% agar-agar were compared. Along with this study, the germination in pure water was tested as well. The results are compiled in the Tables I and II annexed at the end of the paper.

Table I. This set of experiments concerns the following three species, *Dryopteris*, *Woodwardia*, and *Matteuccia*. The table indicates that the addition of agar-agar proves slightly unfavorable for the germination of the first two species and a little more so for the last. For the later development of the germling, however, the effect is

reversed, and the prothallia on a solid medium displayed more rapid growth than in a liquid medium. For instance, the first longitudinal division of the prothallial cell was noticed on the ninth day on the solid medium for all the three species, while those on the liquid medium showed no such division on that day. In water, germination of the spore was not observable even after a lapse of 60 days.

Table II. The general tendency is the same as that of the preceding experiment. The culture medium I is always the most favorable for fhe spore germination. As for the ultimate per cent germination, the addition of agar-agar seems to be of little or no consequence in this case. The fact that a different result was observed in the preceding experiment with *Matteuccia* may be due to the insufficient time of observation, and if the counting had been carried on further, the values in the I and II culture media would approximately be the same. The difference in the further development was not studied here. With respect to the germination in pure water, a positive result was obtained here, in contrast to the foregoing experiment. Evidently there must exist certain specific difference in the germination capability in pure water. LAAGE (1907, p. 101) reports that *Pteris aquilina, Aspidium filix mas, Polypodium dryopteris, Asplenium lucidum* and *Polypodium aureum* are capable of germination even in the conductivity water, while the reverse is true with *Aspidium aculeatum* and *Asp. spinulosum.* Further examples of fern spores capable of germination in pure water are given by WUIST (1910, p. 218) with *Onoclea struthiopteris,* and by ROGERS (1923, p. 75) with *Lygodium palmatum.* The first three species of my study seem to belong, to the latter category of LAAGE. As for the *Osmundaceae* spores, their germination capability in pure water is recognized also by LAAGE (1907, p. 79).

Thus the above two experiments coincide in that the KNOP's solution is the best of the three media tested for the spore germination, and furthermore, it is self-evident that for the purpose of investigating the effects of other factors, it is highly preferable to choose the best condition of medium. On the other hand, however, the instability of the liquid medium makes the focusing of the microscope very difficult, a serious drawback in the procedure of counting under microscope. To get over this difficulty quartz sand impregnated with KNOP's

solution was tested, but to no better purpose, for the unevenness of
the surface makes the focusing equally difficult. For these reasons
the II medium was adopted as the most convenient one for the purpose
of the present study, and in the later experiments, this medium alone
was resorted to for starting the fern spores, except in the case of
special mention.

TEMPERATURE.

To study the effect of temperature, which is one of the most
radical factors of the vital phenomenon as well, is highly important
with regard to the act of germination. But, in contrast to the case
of the phanerogamic seeds, to which innumerable studies are devoted
concerning the matter, rather little attention is paid to the fern spore,
probably because of the less practical importance of the latter; and
we can find only little mention of it in the literature on fern spore
physiology (PERRIN, 1908; KLEBS, 1916, pp. 49-50, 69-71; HARTT,
1925, pp. 433-436).

As it was desirous to get some idea concerning the matter, some
experiments were undertaken, of which brief mention is given below.

The following five species were studied: *Equisetum arvense,
Osmunda japonica, Osm. cinnamomea, Dryopteris viridescens,* and
Woodwardia orientalis.

Of these five species, the spores were prepared after the procedure
described above, and were sown on the standard medium in the
standard germinator, kept at various temperatures, and the rate of
their germination was counted under the microscope. For the source
of illumination, natural diffused light from the window facing northward
was adopted and the germinators were arranged in row, equally distant
(60 cm.) from the window. Except in the experiment of 1928, the
use of more than two thermostats at the same time was not afforded,
so that we cannot make combinations of but two different temperatures
each time, viz., 15°-20°, 20°-25°, 25°-30°, etc., subsequently to compare
their effects all together. Since the intensity of the natural light
employed in the course of the study is quite variable according to
the weather, the light conditions were far from identical in different
sets of experiments. Moreover, as time goes on, the spores suffer

Fig. 1. *Equisetum arvense.*
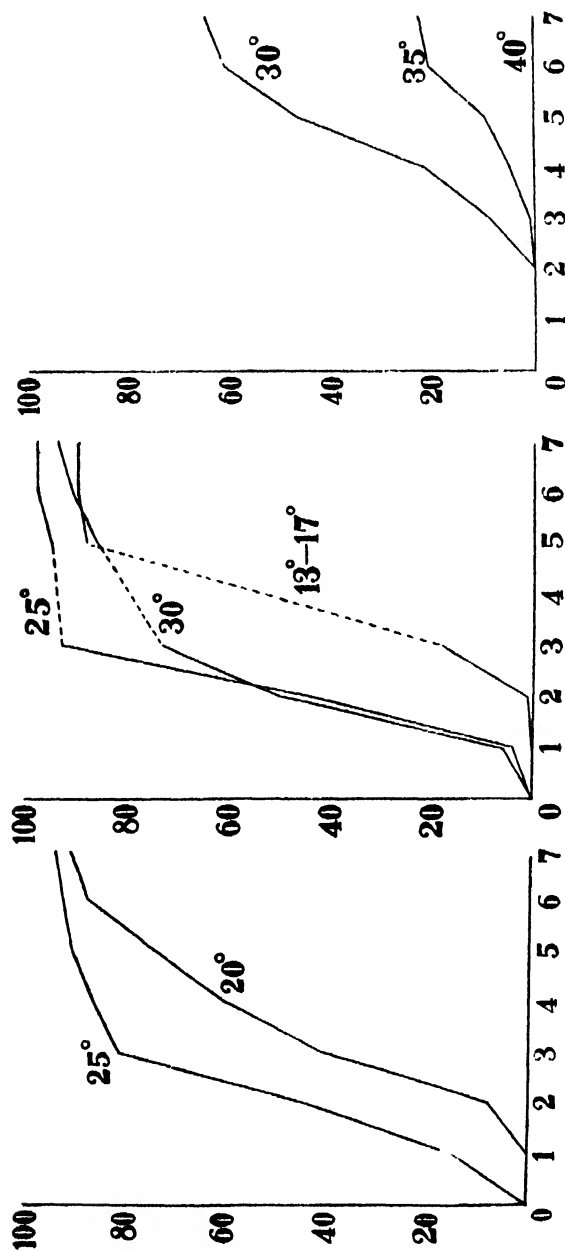
more or less decrement in their vital activity. Mainly from these two causes, the rate of germination does not coincide in different sets of experiments, even if a spore in the same lot and at the same degree of temperature was employed. Therefore the combination of the results of each set of experiments in one common table, which would be of great convenience if possible, was not practicable. Comparing the experiments set by set, however, we can still get some idea of the effects of different temperatures. Later, the experiments with *Woodwardia* and *Polystichum* were conducted at six different temperatures under the same light condition at the same time. Hence the more simplified results concerning these latter two species. In the Tables III-VII at the end of the paper are shown the experimental results, and the graphical representations are plotted on pp. 135-140.

Equisetum arvense. As will be referred to later, the rate of reduction in viability is exceedingly rapid for this species as compared with others. Spores only one week old show so remarkable a reduction in germination capability if sown in an equally favorable condition, that comparison of such results is almost meaningless. Hence the invalidity of the same lot for experiments of different dates. For this reason, in the experiment with *Equisetum*, different lots were used in each set of experiments, freshly collected each time from the same locality. Still the results thus obtained may indicate the general features of the temperature effect. From Table III (and Fig. 1) we know that: 1. Germination can take place up to $35°$. At $40°$ the spores are incapable of germination and turn a brownish colour in a few days, and this point seems to be supramaximal for germination. 2. From room temperature ($13°-17°$) to about $30°$, there was noticed no remarkable difference among the experiments at different grades of temperature, so far as it concerns the ultimate germination per cent. If we take into account the daily progress of germination, however, we can discern the optimal effect at about $25°-30°$. 3. As to the minimal temperature, we were unable to determine it, due to the difficulty of obtaining a constant low temperature, combined with the extremely short life of the spore. *Osmunda japonica* (Table IV and

Figs. 1–5. Effect of temperature on the germination of the fern spore. (The abscissae denote the duration of the experiment in days and the ordinates the per cent of germination.)

Fig. 2. *Osmunda japonica.*
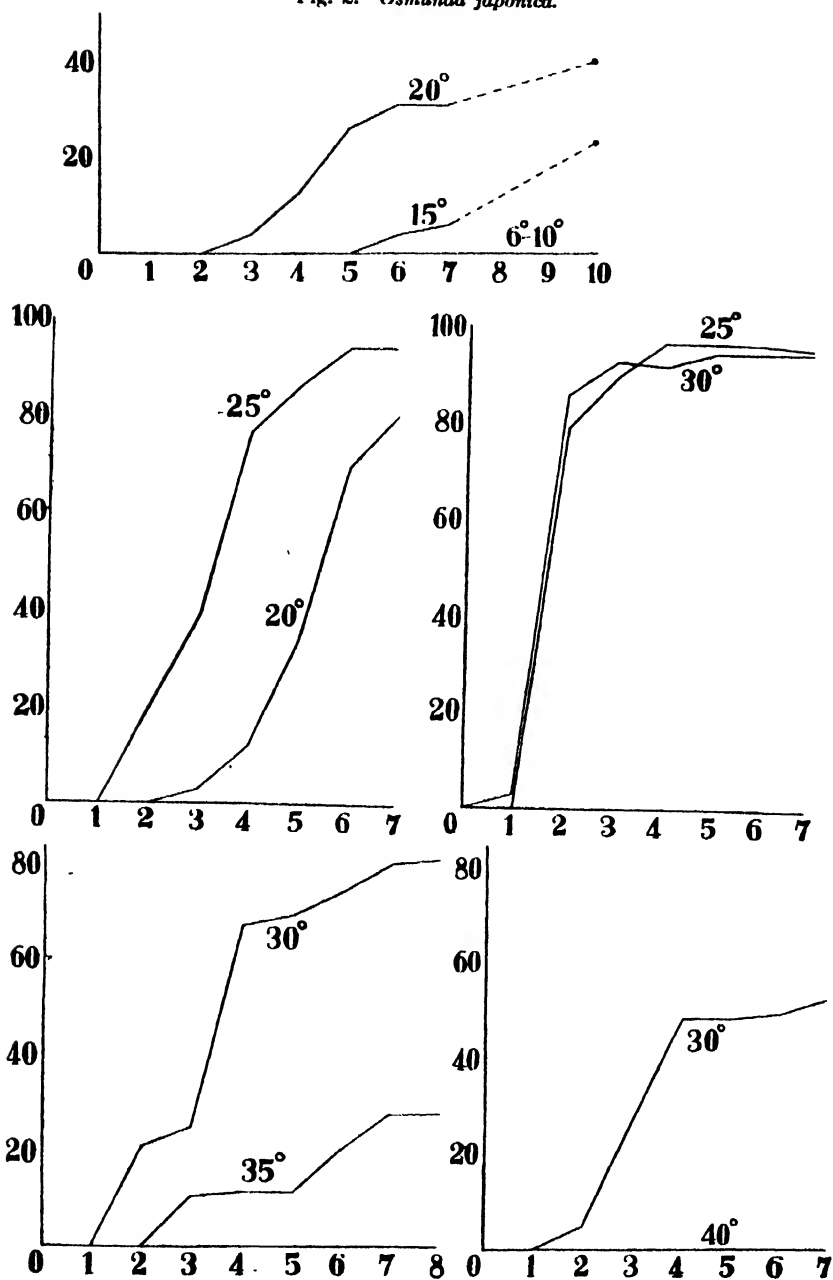
Fig. 3. *Osmunda cinnamomea.*
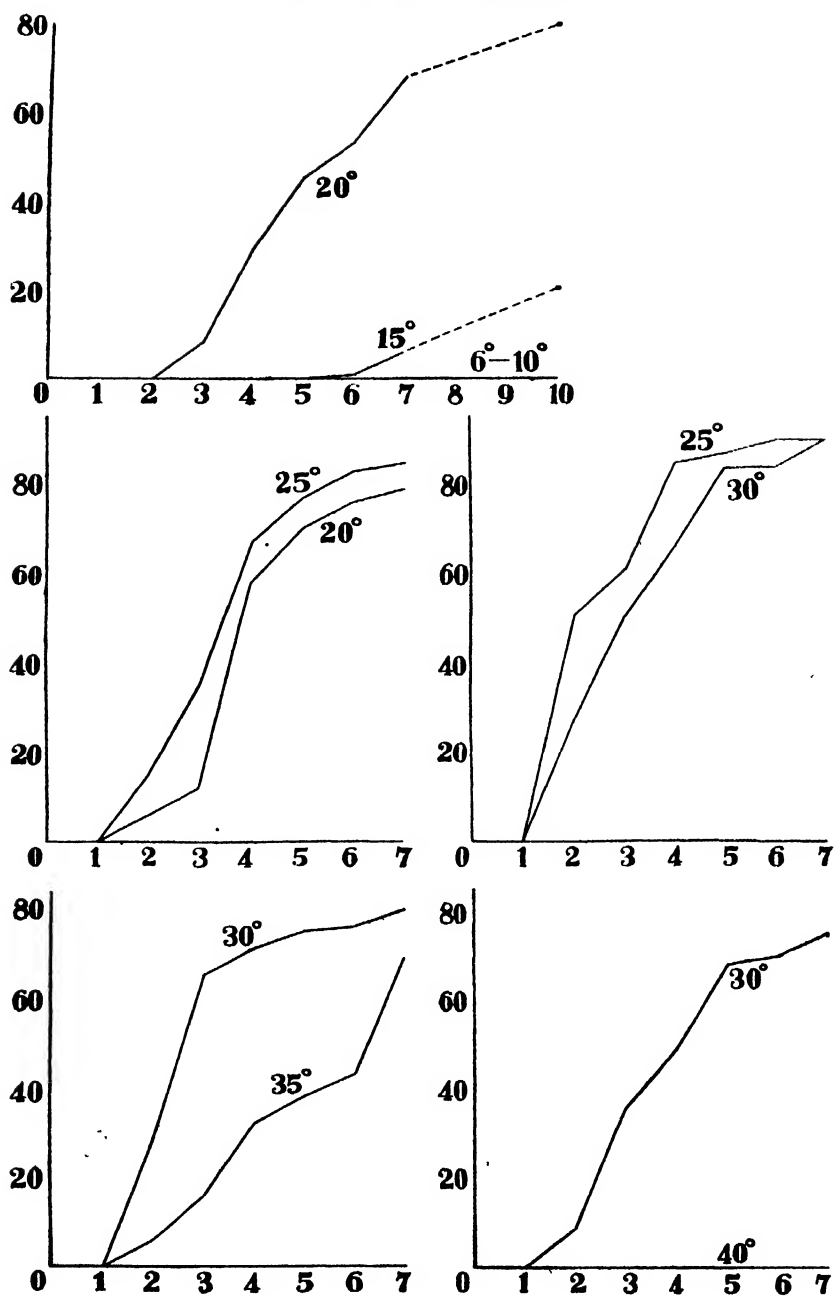
Fig. 2). The optimal temperature for germination is about 25°–30°. Departure from this point, towards both higher and lower temperature, resulted in reducing the rate of germination. The maximal and minimal limit for germination is to be reached at a little lower than 40° and a little higher than 10° respectively. (As we cannot succeed in obtaining a constant low temperature in summer, the experiment to determine the minimal point was conducted in winter, when the age of the spores was more advanced, and their vitality accordingly suffered some decrement. Whether this minimal point holds good also for freshly collected spores cannot be said definitely at present.).

Osmunda cinnamomea (Table V and Fig. 3). For this species the general features are almost identical with the preceding. the optimum being about 25°, subminimum 10°, and supramaximum 40°.

Dryopteris viridescens (Table VI and Fig. 4). For this species and the next, experiments were carried on at six different degrees of temperature under equal light conditions at the same time. Of the

Fig. 4. *Dryopteris viridescens.*
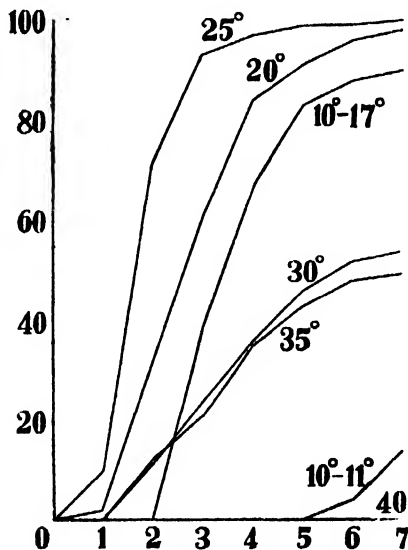
three cardinal points of germination, the maximum lies at about 40°, optimum at about 25°, minimum at lower than 10°. At 10°, we can

still get some spores germinated, though the rate is quite low, so that this point lies still within the limit of the critical point, or supra-minimal. The real minimum point was not determined.

Woodwardia orientalis. As stated above, a parallel experiment to the preceding was carried on for this species. The results are shown in Table VII and Fig. 5, from which we can recognize the optimum

Fig. 5. *Woodwardia orientalis.*
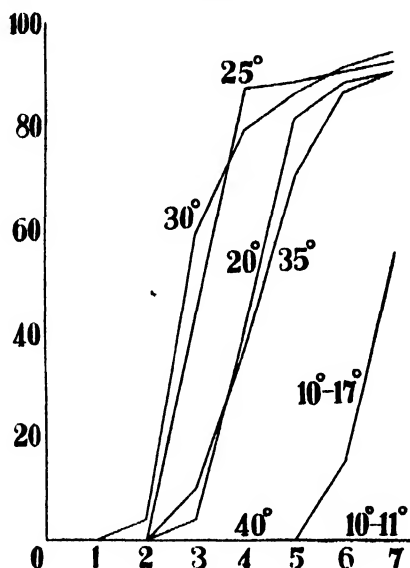
at $25°$–$30°$, the maximum at about $40°$, and the minimum at about $10°$.

The experimental results with the five species studied in the present section will be briefly summarized here. Different as was the behavior in detail, yet the general tendencies are not of fundamental difference among all of these species. With all of the species studied, the optimal point for germination lies at $25°$ or thereabout. According to former workers, the optimal temperature for germination for most species of pteridophytes is found to be at about $25°$, although slight deviations are naturally to be expected. (KLEBS 1916, pp. 49–50, 69–71 ; HARTT, 1925, pp. 434–5).

ILLUMINATION.

The remarkable effect of light on the spore germination as well as on the prothallium formation may be regarded as one of the most interesting and intricate subjects of study in the field of fern spore physiology, and a number of researches have been devoted to this problem up to this date. Thus, the papers by BORODIN (1868), HEALD (1898), BURGERSTEIN (1900, 1908), SCHULZ (1901-2), LAAGE (1907), NAGAI (1914), LIFE (1907), KLEBS (1916, 17), HARTT (1925), and lately by STEPHAN (1928) all treat more or less of the matter in question. Revision of the historical data in detail seems to be without the scope of this study, and further investigation beyond the mere references is omitted here.

As it was desirable to reach some conclusions on the light-relation of the spores of my study before proceeding to investigate their viability, some experiments were undertaken. The species studied were *Equisetum arvense, Osmunda japonica, Osm. cinnamomea* and *Dryopteris viridescens.* As the light source, a gas-filled tungsten filament Mazda lamp of 500 w. 100 v. was used for the most part, and the light emitted was filtered through a water layer in a cuvette 4 cm. wide. The water in the cuvette was incessantly circulating, being freely supplied from the tap. The germinator dishes were set vertically on the laboratory bench on the same level as the light source, so that the surface of the substratum was perpendicular to the horizontal light rays from the lamp, and the lower side of the dish was dipped in water to keep it moist. In this series of experiments, the nutritive medium contained 2% agar-agar, twice as much concentrated as in other cases, for it was found impossible to keep the vertical position otherwise. The germinators were arranged in positions of different distances from the light source, viz., 70, 100, 141, 200, and 282 cm. The relative illumination at each point was therefore 1, 1/2, 1/4, 1/8, and 1/16 approximately, and since the 500 w. gas-filled tungsten lamp emits 625 candles in round numbers (the performance of the lamp was assumed to be approximately 1.25 candles per watt (WALSH, 1923, p. 70.)), the above relative value may be expressed in meter candle as 1250, 625, 313, 156 and 78. Alternating current electricity supplied from the town was employed, which cir-

cumstance made the light intensity rather variable, so that high exactitude cannot be claimed for the above valuation. Actual estimation of the light intensity was not attempted. Along with these series, a parallel germination test in sunlight and with the vacuum-type tungsten filament Mazda lamp of 50 candles was carried out. In this latter case, a thermostat of 25° was employed, and the distance from the lamp was regulated to 40 cm., so that the illumination was reckoned to be roughly 312 mc. The other experiments were all carried on at room temperature. Results of these experiments are shown in Tables VIII–XI.

With the range of the light intensity of the present experiment, the maximum limit of germination could not be determined, nor could the optimal value, except for *Equisetum arvense*, which germinated best in about 625 mc. The other three species have a much higher requirement and the optimal light values seem to be beyond the highest point in the present study, i. e. 1250 mc. of the gas filled tungsten lamp. As for the minimal light value effective enough to cause germination, we will treat of it in connection with the experiments in the next chapter.

In the case of all the four species studied, it is unanimously true that the germination rate in the light of an ordinary tungsten lamp is much higher than in that of the gas filled tungsten filament lamp, notwithstanding the lower light intensity of the former. This is evidently due to the difference in the composition of the light emitted, for the light in the former case contains more red rays than the latter. The favorable effect of the less refrangible rays on the germination is demonstrated by all the authors who treat of the subject (HEALD, 1898, p. 28; SCHULZ, 1901–02, p. 85; BURGERSTEIN, 1908; KLEBS, 1917 a, 1917 b; HARTT, 1925, p. 436; STEPHAN, 1928 pp. 393, 397, 401.).

Briefly speaking, the effect of light on the germination is very remarkable, and the rate of germination largely depends on the light intensity applied. Nevertheless, the range of the light intensity which effects the completion of the then obtainable maximal per cent germination, if due time is allowed, is quite wide. Now, in my experiment of the study of viability, as constancy of the temperature was secured by means of thermostats and the lamp was lit inside them, the use

of a lamp of strong intensity necessarily caused an excessive rise of temperature beyond the optimal point, or we had to manage without strong light but with a favorable temperature condition. Of these alternatives, the latter method was resorted to, and in view of compensating for the weakness of the intensity, the tungsten filament lamp of the vacuum-type was employed, the light of which largely consists of the less refrangible rays.

GERMINATION IN WEAK LIGHT AND IN DARKNESS.

Though of but little consequence to the theme of the present study, the germination of the fern spores in weak light, and further in darkness, was studied in connection with the preceding experiment. Hence the brief account given below.

A) Germination in weak light.

As the light source, a vacuum type tungsten filament Mazda lamp of 5 candles was employed. Filter through the water layer was not applied in this case, as the heating effect was found negligible. Distances from the light source were 1 and 2 meters, or converted into illumination intensity, 5 and 1.25 mc. In Table XII are shown the experimental results from which we know that with light of very low intensity, we can still obtain unmistakably some rate of germination, which property is, however, naturally of specific difference in quantity according to the material used. For instance, spores of *Osmunda japonica* have a much lower requirement than those of *Osm. cinnamomea*, i. e., the former are capable of germination in a light so weak as to be ineffective for the latter. Still much weaker light is sufficient in the case of *Equisetum* and *Dryopteris*. In general, fern spores do not require high intensity of light at all, so far as only the germination is concerned. KLEBS (1916, pp. 23, 76) reports that the spores of *Pteris longifolia* germinate quite abundantly with the osram lamp of below half a HEFNER candle, and the spores of *P. serrulata* have still less requirement for light.

B) Germination in darkness.

Since the work of BORODIN (1868) who first noticed the fact that the spores of some ferns fail to germinate in darkness, the problem has been subjected to the researches of numerous investigators, the

results of which do not, however, always fairly coincide. References to earlier researches are to be found summarized in LAAGE (1907, pp. 76–78), SCHULZ (1901–02, pp. 81–84), and HEALD (1898, pp. 25–27). Furthermore, by NAGAI (1914, pp. 320–321) and later by HARTT (1925, p. 429) are given reviews of the problem in tabular form. The cause of the discrepancy among different authors seems to be of quite a complicated nature. Other conditions which may interfere with the phenomenon in question are numerous; for instance, the culture medium, temperature, viability of the spore, duration of the experiment, the criterion for the germination etc., and minor differences in these factors seem to be effective enough to cause the discrepancy in the results. Taking these circumstances into account, the consistent comparison of the results of former workers with one another is far from facile, nor does it seem appropriate in the present paper to engage in the discussion. I will presently proceed to report on the experimental data in my study.

By way of the experiment on the effect of different light intensity on the spore germination, experiments were carried on to study the behavior of the fern spore sown in absolute darkness. The germinator and the substratum were the standard one previously described. Presently after the sowing of spores, the germinators were wrapped with blackened paper, encased in blackened zinc can, and set in place in the dark thermostat. The apparatus proved to be perfectly light tight. After 7–10 days, when the parallel preparations kept under favorable light condition showed quite high rate of germination, then the germinators in the dark were brought into the light for the first time to be examined under microscope. The results are shown in Tables XIII–XVIII, on which brief comment will be given here.

The spores of *Equisetum arvense* are capable of germination in the dark at room temperature. Application of high temperature resulted in an unfavorable effect. With regard to the spores of this species, most of the former workers agree in the point that positive results are obtained with the germination in the dark (MILDE, 1853; SADEBECK, 1877, p. 45; HEALD, 1898, p. 43). SCHULZ (1901–02, p. 96) reports, however, that he could not obtain any spore of *E. arvense* germinating in the dark. My experiment proved to corroborate the former case so far as the experiments at the room temperature are

concerned. Application of high temperature resulted negatively.

As for the germination capability of the *Osmundaceae* spores in the dark, contradictory results are reported even with the same species. The results of former workers arranged in tabular form are shown below :

Species	Germination	Author
Osmunda regalis	—	KNY, 1872.
,,	—	BURGERSTEIN, 1900.
,,	+	LAAGE, 1907.
,,	+	GOPPERT.[1]
Osm. cinnamomea	—	BURGERSTEIN, 1900.
Osm. Claytoniana	—	,,
Osm. gracilis	+	GOPPERT.[1]

In my study of fern spores along the line of this problem, I have already demonstrated in the preceding section that the spores of *Osm. cinnamomea* show a higher demand for light than those of *Osm. japonica*, and the germination capability of the former is arrested by far the more by the reduction of the light. Still more unfavorable is the absolute darkness, and we cannot find any spore of this species germinated in darkness. *Osm. japonica* has a little less demand for light than the preceding, and by the application of high temperature (25°), germination was attained to some degree. Such a favorable effect of high temperature on the germination in the dark was early recognized by HEALD (1898, p. 43) in the case of *Ceratopteris thalictroides* and *Alsophila Loddgesii*. On the other hand, LAAGE (1907, p. 85) holds a contrary opinion in regard to all of his materials. The difference between my two species of *Osmundaceae* above mentioned with regard to the requirement for light quantity is further proved by the fact that *Osm. japonica* germinated to some degree in case of exposure to mid-day sunlight, one or ten minutes daily for ten days, while *Osm. cinnamomea* failed entirely to germinate in the same condition (Tables XIV–XV).

The spores of *Dryopteris* and *Woodwardia* did not germinate in the dark either at high temperature or at room temperature.

---

[1] cited in LAAGE (1907).

The materials used in the above study, excepting those kept at higher temperatures, were still quite vital at the close of the experiments in the dark, and if kept further under proper light condition to observe their later fate, showed very high per cent of germination. Also, the control preparation, that is, those kept under diffused light from the beginning, showed an equally high percentage. By the way, it may be noticed that in the case of a positive result in the dark, only the first cell divisions were recognized and the typical formation of the rhizoid was not established.

GERMINATION IN ANAEROBIC CONDITION.

The oxygen relation of the germination was repeatedly dealt with in many elaborate papers with regard to the seed, and especially in connection with the question of the delayed germination (SHULL, 1911; GASSNER, 1911; CROCKER, 1906; BIHLMEYER, 1927; BOHMER, 1928). Thus it is generally accepted that an oxygen supply is indispensable for most seeds to germinate (COWLES, 1911, p. 933), though some exceptional cases are also reported (TAKAHASHI, 1905; CROCKER, 1907; CROCKER and DAVIS, 1911; NAGAI, 1916; MORINAGA, 1926; TERASAWA, 1927).

As for the fern spores, far less study has been performed with especial regard to the subject. SCHINDLER (1926) noticed that the fern spores, when sown in liquid medium, show a difference in the rate of germination between those laid on the surface and those sunk below, and he takes the difference in the oxygen supply as responsible for this phenomenon. In my study of the substratum, liquid medium was studied also, but, since the depth of the medium was quite little (about 5 mm.), no difference was noticed between the surface and the bottom. If, however, the oxygen supply is entirely arrested, a remarkable influence can be observed. The fern spores seem to be incapable of germination under absolutely anaerobic condition. The fact is proved by the following experiments (TABLE XIX). Spores were sown in the germinator with the standard substratum and the germiantors themselves were placed on the middle shelf in a glass vessel with plain glass top. The capacity of the vessel was about 2000 cc. At the bottom of the vessel were put beforehand 25% pyrogallol 1 volume and 6% NaOH 6 volume in separate containers.

After the air in the vessel was replaced with hydrogen gas, the chemicals were mixed together, which is sure to absorb the trace of oxygen if any (MAYER, 1903. p. 148). The experiments were conducted either in daylight or with artificial light in a 25° thermostat. The materials used were *Equisetum arvense, Osmunda japonica, Osm. cinnamomea, Dryopteris viridescens,* and *Woodwardia orientalis.* All of them failed to germinate in the anaerobic condition, so long as the duration of the experiment did not exceed one week, which is sufficient time to get the full germination per cent under aerobic condition. As for the proof that the materials were quite viable, the per cent of germination in the aerobic condition may be consulted, which is noted also in the table. The preparations, after remaining one week under the anaerobic condition, were exposed to a free supply of air in the laboratory. After one week in the new condition, the rate of the germination was counted again, which results are given in column 6, showing that the *Equisetum* and *Dryopteris* spores are far less resistant than the others.

II. ON THE VIABILITY OF THE FERN SPORE.

So far we have dealt with some of the features of the germination physiology of the fern spore with respect to external conditions. We will now proceed further to the question of their viability.

Taking into acccount the fundamental function of the spore, I have adopted the germination capability as the index of the viability. Spores were stored under various conditions after they were collected from the fields, and tested for the rate of germination from time to time. As for the conditions for germination, the knowledge obtained in the preceding sections was consulted. It is obvious that whether a spore is potent to germinate or not must not be decided with experiments under unfavorable conditions. To take an extreme case, if we sow the spores under a bad condition, say, in a dark, cold place without oxygen supply, they are sure to fail to start germination though potent enough if placed under a better condition. Hence the imperative necessity to afford the optimal condition in such an experiment. Now, we have studied in the preceding sections about some of the more important factors in germination, viz., substratum,

temperature, illumination and oxygen supply, and learned roughly the general tendencies. To combine all of the optimal conditions was, however, unable to be attained in practice. For, as a matter of fact, we cannot afford to prepare different conditions for each different species. Moreover, the thermostat available had to be illuminated with a light source inside, which naturally made it impracticable to combine high light intensity with rather low temperature. Limited by these circumstances, we were compelled to resort to as optimal conditions as practicable, if not absolutely so. Thus we applied the temperature of 25° for all of the species, since this temperature is almost equally favorable to all of them. As a light source the vacuum type tungsten filament Mazda lamp of 50 candles was employed and the germinators were fixed at a distance of 40 cm. from the light source. The favorable effect of this arrangement as compared with the gas filled tungsten lamp was demonstrated in the earlier part of this paper. 0.1% KNOP's solution added with 1% agar-agar was used as culture medium, which though a little inferior to those without agar-agar, is almost equally effective for obtaining the ultimate per cent of germination. As for the supply of oxygen, no positive contrivances were attempted, but considering the retarding effect of oxygen deficiency, attention was paid to prevent the cover of the dish from fitting too tightly, so that free communication of air over the surface of the substratum was secured. The germinator vessels and the procedure of counting the per cent of germination were the same as in the preceding experiments. The experiments were carried on until the germination per cent was reduced to nil, and the spores in the germinator vessels were discolored away. This critical point was assigned as the limit of the span of the life. Four species mostly were studied, viz., *Equisetum arvense, Osmunda japonica, Osm. cinnamomea*, and *Dryopteris viridescens*, and in some cases, studies were extended to *Woodwardia orientalis* and *Matteuccia struthiopteris* as well. Four different aspects were considered with respect to the present problem. First, specific difference in the longevity of the spores; secondly, the effect of different methods of storage; thirdly, the interrelation between the specific difference in the longevity and the other properties of the spores; and finally, the relation of viability decline to the change in the catalase content.

SPECIFIC DIFFERENCE IN THE LONGEVITY OF THE SPORE.

Experiments on this point was carried on in 1925, but with no special regard to observe the strictly uniform conditions for germination, so that no absolute value can be claimed for the obtained results. It seems nevertheless valid enough to indicate that the difference in the longevity of the spore due to the species is quite remarkable. Therefore, the extract from the protocol concerning the per cent of germination of the spores is given in Tables XX, 1-6. In this set of experiments, preliminary study with regard to the keeping quality of different storage methods was also undertaken. As the matter is, however, to be treated of in detail in the succeeding chapter, mention will be limited here to those spores kept in the ordinary condition of the laboratory, packed in paraffin paper sacks exposed to diffused light from the north window, and having temperature and humidity controlled in no way.

Here are summarized from the tables the time-limits of the spores of the six species when kept under such a condition:

Equisetum arvense,	10–24 day
Osmunda japonica,	23–43 „
Osm. cinnamomea,	43–54 „
Dryopteris viridescens,	92 107 „
Matteuccia struthiopteris,	138–149 „
Woodwardia orientalis,	174–191 „

Remarkable is the extraordinary brevity of life of the *Equisetum* spore, which property was naturally often noticed by former workers who engaged in the study of this species (MILDE, 1851, p. 621; DUVAL-JOUVE, 1864, p. 95; SCHULZ, 1901-02, p. 98; STEPHAN, 1928, p. 399; GISTL, 1928, p. 257). *Osmunda cinnamomea* and *Osm. japonica*, too, lose their viability after a comparatively short period. This fact is also coincident with the observations in the former researches on *Osmundaceae* spores (SADEBECK, 1881; LAAGE, 1907, p. 82). *Dryopteris, Matteuccia* and *Woodwardia* come next in the order. Of the six species studied, *Woodwardia* was the most long lived, but even with this one, the span of life was nevertheless observed not to exceed 191 days. It must be kept in mind, however, that these numerals concern only those spores which were kept in the ordinary condition

of the laboratory, with temperature and moisture regulated in no way, and of which absolute exclusion of the light was not aimed at, as they were only packed in paraffin paper sacks. Different conditions may greatly alter the value, for instance, storage in the dark and in a dry condition is far more favorable for keeping the viability. We can find in literature, examples of the long life of fern spores (SADE-BECK, 1881 ; COLELY and DRUERY, 1904, p. 13 ; WRIGHT, 1909), most of which seem to owe their longevity to the storage in a dark condi-tion, though the specific character must be at least equally respected.

EFFECT OF DIFFERENT METHODS OF STORAGE AND THE REDUCTION OF THE VIABILITY DUE TO AGE.

In the course of the preceding experiment, the effect of different methods of storage was preliminarily studied, which yielded some cases of interest. In 1926, accordingly, further studies with special respect to this problem were undertaken. Four species of comparatively short life, viz., Equisetum arvense, Osmunda japonica, Osm. cinnamo-mea, and Dryopteris viridescens were applied as materials of the study, and three kinds of factors, light, temperature, and moisture were studied.

In connection with the light relation, the effect of direct sunlight, the same filtered through red glass, the same filterd through blue glass, diffused sunlight in the laboratory, and lastly storage in darkness were compared. As for the temperature relation, storage in a 25° thermostat, in the ordinary room temperature of the laboratory, and in a refrigerator (MANNESMANN's electric refrigerator was employed, in which the normal range of temperature was (−2°)–4° for the winter and 5°–10° for the summer), and in a receptacle immersed in the brine of the ice manufactory (−11° to −16°) were compared. Finally, with regard to the moisture relation, the effect of desiccation (exsic-cator with 1/4 total volume filled with $CaCl_2$ was employed) as contrasted to the storage in the ordinary room humidity, was studied. In studying the effect of exposure to the light, the spores were spread over the paraffin paper in a PETRI dish, so that an overshadowing of each other was prevented. In other cases, small tube bottles were employed as the receptacles. Various combinations of the above three factors were studied, except what was impracticable, for instance, we

were unable to study the low temperature combined with exposure to light, as the refrigerator admits no illumination. At any rate, the results obtained do not fail to show, to some degree, the general features concerning the problem. As for the conditions for the germination study, those given in the previous part (p. 148) were strictly observed throughout.

Protocols of the experiments are compiled in Tables XXI-XXIV.

We can perceive from these tables the general tendency that a dark, cold, and dry condition seems to be more favorable than a light-exposed, warm, and moist one. In *Equisetum*, slight deviation from this general tendency was encountered concerning the moisture-relation in the higher temperature. This species is, however, not quite appropriate for studying these problems, as it is excessively short-lived, so that whether the discrepancy is idiosyncratic in reality or not, could not be decided clearly.

Red light seems to be more destructive than blue in the case of *Equisetum* and *Dryopteris*, while a reverse result was obtained with *Osmunda japonica*, and lastly, no distinction was observed with *Osmunda cinnamomea*. Concerning this point, however, my experiments were not at all conclusive, as direct sunlight, even after the transmission through the color filter, is too intensive to afford a comprehensive resolution of the two with respect to their destructive effects. Therefore, further study is necessary to resolve the distinction. Storage in Hydrogen gas yielded no remarkable effect as compared with that in ordinary air. In all of my material, reduction of viability due to the age is clearly demonstrated (A few anomalous cases which are probably due to some uncontrollable variation in the conditions for germination could not be excluded.), which property seems to be shared by almost all of the fern spores (LAAGE, 1907, p. 82; ROGERS, 1923, p. 76; STEPHAN, 1928, p. 389).

DIFFERENCE IN VIABILITY AS RELATED TO OTHER PROPERTIES.

In the preceding chapters we have reported on the comparison of the longevity of the fern spore, the decline of viability with age, and furthermore, the influence of external conditions on their keeping

capacity.   Now, it naturally follows that these differences in viability of the spore must necessarily be correlated with their physico-chemical constitution.   In the present section, accordingly, some properties of the spore are investigated and brought into comparison with one another to see whether they have some parallel relationship to the question of their viability.

i.   Water content of the spore.

In general, the fern spore collected freshly shows far less value in its water content than the vegetative cells.   These values are to some degree comparable to the water content of the seed of the Phanerogam, which is reckoned to be 10% or thereabout (SKENE, 1924, p. 399).   The actual values measured are shown in Table XXV.

On inspection of the table, we can notice that these fern species, when arranged in order according to their water content, perfectly coincide with the order in the table on page 149, which represents the comparison in length of their span of life.   The fact is interesting in particular that the *Equisetum* spore which is remarkable for its extraordinary short life, contains a far larger amount of water than the others, having half of its weight consisting of water.

Generally speaking, the scanty water content is accounted for as the characteristic of seeds and spores, because the protoplasma of such composition is much more resistant against hard conditions.   The fact that the *Equisetum* spore contains a larger amount of water than the others suggests that it represents rather a fragment of a vegetative body than an ordinary disseminule, and their unusual brevity of life may be at least partly due to this property, although the other properties, such as the nature of the spore wall and that of the reserve substances as suggested by SCHULZ (1901-02, pp. 94, 96) are also responsible.   The same principle may be applicable to the case of the *Osmundaceae* spores, that is, in this case also, the abundance of the water content, besides other characters noticed by KNY (1872, p. 3) and LAAGE (1907, p. 83), is distinct in contrast to the other longer-lived species.

ii. Respiration intensity of the spore.

We have above just learned of a remarkable similarity between the spore and the vegetative cell of *Equisetum* as contrasted to other species, with respect to the water content, one of the fundamental factors in the cell constitution. It seems, accordingly, highly probable that the *Equisetum* spore exhibits activity in vital phenomenon similar to a vegetative cell, while the reverse is the case with other species. The fact is fairly demonstrated with the phenomenon of respiration, about which, the result of experiment will be reported below.

The materials used were the following five species, *Equisetum arvense, Osmunda japonica, Osm. cinnamomea, Dryopteris viridescens,* and *Woodwardia orientalis.* As the container for the spore a wide-mouthed glass bottle of about 200 cc. capacity was employed. 3 to 4 g. of the material was put into the receptacle, lowered into the constant temperature water bath with electric heater and regulator, and a current of CO_2 free air was constantly supplied. The CO_2 produced in the respiration was determined by means of the volumetric method in the usual manner.

The duration of the experiment was usually 6–8 hours. In the Table XXVI, the CO_2 gas developed is expressed in mg. per 24 hours per 1 g. of the spore material.

From the table we can perceive clearly that the *Equisetum* spore far exceeds the others in the respiration intensity, the values for the latter being mostly under the limit of the experimental error, even at the higher temperature. Only the spore of *Woodwardia* exhibits a slight sign of respiration. More remarkable is the contrast at the lower temperature, for, at 15°, only the *Equisetum* spores were seen to respire, all the others being devoid of the capability. As for the *Equisetum* spores, the respiration intensity at 25° is roughly twice as large as at 15°. The decline of the respiration intensity with age is also demonstrated in the table with the *Equisetum* spore.

Thus we can establish here again a close relationship between the brevity of life and other physiological features of the *Equisetum* spore. But, as to whether this principle may be applicable further to the longevity of other species cannot be decided, as the respiration intensity of all the other species is too small to allow a consistent comparison.

iii. Catalase content of the fern spore.

The physiological significance of the catalase has been discussed by many authors, who do not reach a coincident conclusion. Opinion prevails, however, that the enzyme plays an important rôle in connection with the metabolic activity, and the matter has been often treated in the physiology of the seed. To determine the same with the fern spores, the writer carried on some experimental studies, of which the results are given below.

Measurement of the catalase activity was performed by means of APPLEMAN's method[1] (APPLEMAN, 1910, p. 184, 1916, p. 226; CROCKER and HARRINGTON, 1918, p. 139) at 25° and rocking 60–80 times per minute. The spores were ground in a glass mortar, to which was added 10 cc. Aqua and a small quantity of Ca-carbonate. 10 cc. of H_2O_2 (about 3%) was always given. Quantities of the spore used were not constant throughout these studies, but were arranged roughly to be inversely proportional to the activity of the catalase. In the accompanying tables, the average value for two or three measurments is given, the activity being represented by the volume of O_2 evolved in cc. per 0.1 g. of the fresh or dry weight of the material. There are annexed also in the table the dry matter content and the germination per cent of the spore as well. (For determining the per cent of germination, the condition given on page 148 was observed throughout.)

a) Specific difference in the catalase content.

Five species were studied; *Equisetum arvense*, representing the most short-lived spore and the others somewhat longer-lived. The results obtained are shown in Table XXVII. From these values in the table we can learn but little concerning the relation of the catalase activity to the problem of longevity. Remarkable is the fact that *Osmunda japonica* and *Osm. cinnamomea*, the two closely related species, show a marked similarity both in the longevity and the catalase content as contrasted with other species.

---

[1] An improvement has been lately introduced by OVERHOLSER (1928, p. 286).

b) Reduction due to age.

The catalase content of the spore is not stationary at all through the life of the spore but decreases constantly, the course of decline being naturally influenced largely by the environmental condition or the method of storage. We will now treat of these lines of the problem, and first with regard to the age of the spore kept in a certain definite condition. For this purpose, spores of *Equisetum arvense*, *Osmunda cinnamomea* and *Osm. japonica* were utilized as materials of the study. As for the storage condition, uniformity of method for all the species was not observed, as the keeping properties of the spores are specifically somewhat different (p. 151). Although storage in a dark cold place is more favorable for all of them than in an ordinary laboratory room, they behave themselves somewhat differently with respect to moisture conditions. So that in the present case, to keep their viability as well as possible, we have applied the storage in dark cold, and non-dry condition for *Equisetum*, and storage in a dark, cold, and desiccated condition for *Osmundaceae*. Catalase activity was determined from time to time after the method described above, and at the same time the per cent of germination was counted. The obtained results are tabulated in Tables XXVIII–XXX. They clearly demonstrate that the catalase activity coincides with the germination capability in the general tendency that they both suffer from gradual reduction as their age increases.

c) Effect of the different storage methods.

We have already learned in regard to the catalase content of the spore that it does not remain stationary during the period of storage but is gradually reduced in its activity. The rate of reduction is, in its turn, not uniform in all cases. On the contrary, it is largely influenced by the conditions given by the storage method, as is the case with the viability of the spore. A few experiments were undertaken with respect to this question with the same three species as in the preceding section. As for the conditions in the storage methods, two factors were subjected to study, viz., temperature and moisture. As is previously described on page 150, concerning the conservation of

viability, high versus low temperature, and a dry versus non-dry condition were investigated, and the same methods used there were applied here as well. Different effects of other factors were not studied. Experimental data obtained with the above three species are shown in Tables XXXI–XXXIII. Examining these tables, however, we can perceive no definite tendency of general applicability. The effect of a certain single factor on the conservation of the catalase content of the spore differs according to other environmental factors and the material used. Thus the dry storage is favorable for *Equisetum* spore at low temperature while the reverse is the case with the same spores at high temperature. Another example of complication is shown by the spore of the *Osmunda cinnamomea*, for which, in a dry condition, high temperature is more favorable for the conservation of the catalase, while in a non-dry state, the low temperature keeps its activity the better. Briefly speaking, the relation seems to be too intricate. The properties of the spore constitution must each be provided with idiosyncratic features.

We have learned elsewhere that between the storage condition and the keeping capacity of the viability of the spore, there is a certain tendency of general applicability to some extent. In the present tables, we have attached also the experimental data concerning this line of the matter, being represented in the lowermost row of the tables in the form of the per cent of germination. Taking these figures into account, we can compare at the same time the keeping capacity of the catalase content and that of the germination capability with respect to the storage method. The result of the comparison proves to be quite confused, and consistent relationships between these two features cannot be established so far as my experimental results are concerned.

d) *Difference due to treatment.*

So far we have been dealing with the catalase activity, with the spore materials ground fine in the mortar. Modification of the procedure naturally leads to different results. The following experimental data are reported here as an example of this truth. In the course of our study concerning the catalase activity, comparison of the oxygen-

development from the ground spore and the intact spore was undertaken, the results of which are to be found in Tables XXXIV–XXXVI. The experiments usually proved quite remarkable differences between these two, the magnitude of the difference being, in turn, dependent on the species and the age of the spore. Thus we can distinguish quite a favorable effect of grinding with the fresh spore of *Equisetum*, but a little later, when the spore is only 13 days old, the favorable effect is largely reduced, and still later, with a 1 year old spore, almost no distinct difference is to be noticed. As for the *Osmundaceae* spores, the favorable effect of grinding is distinguished with spores both fresh and old, although in the latter case the distinction is diminished to some degree.

The accelerating effect of grinding may be attributed partly to the increase of the working surface of contact between the plasma and hydrogen peroxide, and partly to the destruction of the spore wall. Now the fact that this favorable effect decreases as the days go on suggests either that the interference due to the spore wall is diminished, or that the catalase in the more interior part of the spore suffers from reduction more rapidly. Which of the two possibilities is the more probable we cannot determine. At any rate, the specific difference cannot be neglected in either case if we compare the results on *Equisetum* with those on *Osmundaceae*.

Résumé.

1. The present work is concerned mainly with the following two aspects of the physiology of fern spores, namely, the question of the influence of the external conditions on the germination, and the viability of the spore. The former question is treated in Part 1 of the present paper and the latter in Part 2.

2. The following five species of Pteridophyte were mostly employed as the materials for study, namely, *Equisetum arvense* L., *Osmunda japonica* THUNB., *Osm. cinnamomea* L., *Dryopteris viridescens* O. KUNTZE, and *Woodwardia orientalis* SWARTZ. In a few cases, studies were extended to *Matteuccia struthiopteris* (L.) TODARO.

3. Viability of the spore was represented by the germination capability and those spores which exhibit the first cell division in the

germinator were taken as being capable of germination.

4. Of the three substrata studied (0.1% KNOP's nutritive solution, the same with 1% agar-agar added, and distilled water), the first one always proved the most favorable for germination. But so far as the ultimate per cent of germination is concerned, the distinction was practically negligible between the first and the second. For the later development, that is, the growth of the prothallia, the second was preferable. As for the germination in the pure water, specific differences were noticed. While *Dryopteris*, *Woodwardia* and *Matteuccia* were incapable of germination in pure water, two species of *Osmundaceae* showed some rate of germination, though it was not very striking.

5. With respect to the temperature, the five species studied did not exhibit any remarkable variation. They all agreed in the general tendency that they have the optimum at about 25°, maximum a little lower than 40°, and minimum at about 10°.

6. Germination tests with respect to the illumination were carried on. The results show that rather high intensity is favorable for germination when the light from the gas-filled tungsten-filament lamp is applied. Neither the maximal intensity of illumination to effect the germination nor the optimal value was able to be determined, except for *Equisetum*. For *Equisetum*, the optimum was found at 625 mc. or thereabout.

7. Illumination of lower value than the optimum is still effective enough to obtain full per cent of germination, provided a sufficient time of application is allowed.

8. The light of vacuum-type tungsten-filament lamp was far more favorable than the gas-filled one.

9. The Effect of sunlight was studied in some cases, which proved that the diffused light is quite favorable, while the direct rays are fatal.

10. In very low intensity of illumination, most of the spores studied were capable of germination. Only in one case, that is, the spore of *Osmunda cinnamomea*, was the minimal value for germination attained. The spore of this species did not germinate in illumination lower than 1.25 mc. of the vacuum-type tungsten filament lamp.

11. Spores of *Equisetum arvense* and *Osmunda japonica* were proved capable of germination in the dark, the former at the room

temperature and the latter at 25°. Spores of *Osmunda cinnamomea*, *Dryopteris viridescens* and *Woodwardia* did not germinate in the dark, either at the room temperature or at higher temperature.

12.   In absolutely anaerobic condition, all the spores failed to show any sign of germination.

13.   The life of the spore kept in the laboratory was studied. The span of life was different specifically. The following values were obtained in my study: *Woodwardia*, 174-191 days; *Matteuccia*, 138-149 days; *Dryopteris*, 92-107 days; *Osmunda cinnamomea;* 43-54 days, *Osmunda japonica*, 23-43 days, and *Equisetum arvense*, 10-24 days.

14.   The storage in a dark and cold condition was more favorable for keeping the viability than a light-exposed and warm one. This tendency seemed to be generally applicable. As for the moisture relation, the spores of most species agreed in preferring the dry storage, while it was not the case with *Equisetum* spores at a higher temperature.

15.   Red light seemed to be more destructive than blue in the case of *Equisetum* and *Dryopteris*. The case is reversed with *Osmunda japonica*, and no remarkable distinction can be observed with *Osmunda cinn momea*.

16.   Storage in Hydrogen gas yielded no remarkable effect as compared with that in the ordinary air.

17.   The water content of the spores of my study varies from 5.9% to 49.18% according to the species. Quite remarkable is the close relation of the amount of the water content and the span of life. The *Equisetum* spore which is noted for its brevity of life is also peculiar in its abundant water content.

18.   *Equisetum* spores are idiosyncratic also in the fact that they alone show a decided sign of respiration.

19.   Catalase content of the spores was determined and their reduction due to age was demonstrated.

20.   The relation of temperature and moisture on the conservation of catalase activity was studied, only to attain too complicated results to make generalization possible.

LITERATURE CITED.

APPLEMAN, C. O., 1910, Some observations on catalase. Bot. Gaz., Vol. 50, pp. 182-192.

APPLEMAN, C. O., 1916, Relation of oxidase and catalase to respiration in plants. Am. Journ. Bot., Vol. 3, pp. 223-233.

BIHLMEYER, M., 1927, Der Einfluss der Vorquellung und der Samenschale auf die Keimung lichtgeförderter Samen. Jahrb. f. wiss. Bot., Vol. 67, pp. 702-732.

BOHMER, K., 1928. Die Bedeutung der Samenteile für die Lichtwirkung und die Wechselbeziehung von Licht und Sauerstoff bei der Keimung lichtempfindlicher Samen. Jahrb. f. wiss. Bot., Vol. 68, pp. 544-601.

BORODIN, J., 1868, Über die Wirkung des Lichtes auf einige höhere Kryptogamen. Bull. de l'Acad. Imp. Sci. de St. Petersbourg. Vol. 12, pp. 432-438. (cit. HEALD).

BURGERSTEIN, A., 1900, Keimen Farnsporen bei Lichtabschluss? Bot. Zentrbl., Vol. 88, p. 105, 1901.

BURGERSTEIN, A., 1908, Einfluss des Lichtes verschiedener Brechbarkeit auf die Bildung von Farnprothallien. Ber. D. Bot. Ges., Vol. 26 a, pp. 449-451.

CLEMENTS, F. E., 1907, Plant physiology and ecology.

COLELY and DRUERY, 1904, Longevity of fern spores. Gard. Chron., Vol. 35, p. 13. (JUST's Bot. Jahresber., 32 Jahrg. p. 1055.).

COWLES, H. C., 1911, A textbook of botany, Vol. 2, Ecology.

CROCKER, W. 1906, Rôle of seed coats in delayed germination. Bot. Gaz, Vol. 42. pp. 265-291.

CROCKER, W., 1907, Germination of the seeds of water plants. Bot. Gaz., Vol. 44, pp. 375-380.

CROCKER, W. and W. E. DAVIS, 1911, Delayed germination in seed of *Alisma plantago*. Bot. Gaz., Vol. 58, pp. 285-321.

CROCKER, W. and G. T. HARRINGTON, 1918, Catalase and oxidase content of seeds in relation to their dormancy, age, vitality and respiration. Journ. Agr. Res., Vol. 15, pp. 137-174.

DUVAL-JOUVE, J., 1864, Histoire naturelle des *Equisetum* de France.

GASSNER, G., 1911, Vorläufige Mitteilungen neuerer Ergebnisse meiner Keimungsuntersuchungen mit *Chloris ciliata*. Ber. D. Bot. Ges., Vol. 29, pp. 708-722.

GISTL, R., 1928, Gestalt und Lage der ersten Teilungswand in Sporen von *Equisetum* in Lösungen von verschieden osmotischen Druck kultiviert. Ber. D. Bot. Ges., Vol. 46, pp. 244-266.

GUPPY, H. B., 1912, Studies in seeds and fruits.

HARTT, C. E., 1925, Conditions for germination of spores of *Onoclea sensibilis*. Bot. Gaz., Vol. 79, pp. 427-39.

HEALD, F. DE FOREST, 1898, Conditions for the germination of the spores of bryophytes and pteridophytes. Bot. Gaz. Vol. 26, pp. 25-45.

KLEBS, G., 1916, Zur Entwickelungs-Physiologie der Farnprothallien. I Teil. Sitzb. Heidelberg. Akad. Wiss., Abt. B, 4. Abh. pp. 1-82.

KLEBS, G., 1917 a, Zur Entwickelungs-Physiologie der Farnprothallien. II Teil. Sitzb. Heiderlberg. Akad. Wiss., Abt. B, 3. Abh. pp. 1-138.

KLEBS, G., 1917 b, Zur Entwickelungs-Physiologie der Farnprothallien. III Teil. Sitzb. Heidelberg. Akad. Wiss., Abt. B, 7. Abh. pp. 1-104.

KNY, L., 1872, Beiträge zur Entwicklungsgeschichte der Farnkräuter. I. Entwicklung des Vorkeimes von *Osmunda regalis* L. Jahrb. wiss. Bot. Vol. 8, pp. 1-15.

LAAGE, A., 1907, Bedingungen der Keimung von Farn- und Moossporen. Beiheft Bot. Zentrbl., Vol. 21, Abt. 1, pp. 76-115.

LANG, W. H., 1898, On apogamy and the development of sporangia upon fern prothallia. Phil. Trans. Roy. Soc. London, B, Vol. 190, pp. 189-238.

LIFF, A. C., 1907, Effect of light upon the germination of spores and the gametophyte of ferns. Ann. Rpt. Mo. Bot. Garden, Vol. 18, pp. 109-122.

MEYER, A., 1903, Practicum der botanischen Bakterienkunde, p. 148.

MILDE, J, 1851, Zur Entwicklungsgeschichte der *Equiseten*. Nov. Act. Acad. Caes. LEOP. CAROL. naturae curiosorum. Tom. 20, Pt. 2, pp. 613-636. (cit. DUVAL-JOUVE, 1864).

MILDE, J., 1853, Beiträge zur Kenntniss der *Equiseten*. Nov. Act. Acad. Caes. LEOP. CAROL. naturae curiosorum. Tom. 23, Pt. 2, pp. 557-612. (cit. DUVAL-JOUVE, 1864).

MORINAGA, T., 1926, The favorable effect of reduced oxygen supply upon the germination of certain seeds. Amer. Journ. Bot., Vol. 13, pp. 159-166

NAGAI, I., 1914, Physiologische Untersuchungen über Farnprothallien. Flora, Vol. 106, pp. 281-330.

NAGAI, I., 1915, On the influence of nutrition upon the development of sexual organs in the fern prothallia. Journ. Coll. Agr. Imp. Univ. Tokyo, Vol. 6, No. 2, pp. 121-164.

NAGAI, I., 1916, Some studies on the germination of the seed of *Oryza sativa*. Journ. Coll. Agr. Imp. Univ., Tokyo, Vol. 3, pp 109-158

OVERHOLSER, E. L., 1928, A study of the catalase of the fruits of pear varieties. Am. Journ. Bot., Vol. 25, pp. 285-306.

PERRIN, G., 1908, Influence des conditions extérieures sur le développement et la sexualité des prothalles des *Polypodiacees*. C. R. Ac. Sci. Paris, Vol. 117, pp. 433-435. (JUST's Bot. Jahresber. 37 Jahrg. 2 Abt. pp. 736-7).

ROGERS, L. M., 1923, Development of the prothallium of *Lygodium palmatum* Bot. Gaz., Vol. 75, pp. 75-85.

SADEBECK, 1877, Über die Entwickelungsgeschichte der Prothallien und die Embryologie der Schachtelhalme. Bot. Zeit. Vol. 35, pp. 41-46

SADEBECK, 1881, Die Gefässkryptogamen. (SCHENK, Handl uch der Botanik, Breslau,) (cit. LAAGE).

SCHULZ, N., 1901/02, Über die Einwirkung des Lichtes auf die Keimungsfähigkeit der Sporen der Moose, Farne und Schachtelhalme Beiheft Bot. Zentrbl, Vol. 11, pp. 79-97.

SCHINDLER, E., 1926, Über den Einfluss äusserer Bedingungen auf die Wachstumsweise der Prothallien einiger Farne. Bull. Acad. Polon, Sér. B. Krakau (1925) (Bot. Zentrbl., Vol. 9, p. 361, 1927).

162 Y. OKADA

SHULL, CH. A., 1911, The oxygen minimum and the germination of *Xanthium* seeds. Bot. Gaz., Vol. 52, pp. 453–477.

SKENE, M., 1924, The biology of flowering plants.

STEPHAN, J., 1928, Untersuchungen über die Lichtwirkung bestimmter Spektralbezirke und bekannter Strahlungsintensitäten auf die Keimung und die Wachstum einiger Farne und Moose. Planta, Vol. 5, pp. 381–443.

TAKAHASHI, T., 1905, Is germination possible in absence of air? Bull. Coll. Agr., Imp. Univ., Tokyo, Vol. 6, pp. 439–442.

TANSLEY, A. G., 1923, A practical plant ecology.

TERASAWA, Y., 1927, Experimentelle Studien über die Keimung von *Trapa natans* L., Bot. Mag., Tokyo, Vol. 41, pp. 581–587.

TWISS, E. M., 1910, The prothallia of *Aneimia* and *Lygodium*. Bot. Gaz., Vol. 49, pp. 168–181.

WALSH, J. W. T., 1923, Elementary principles of lighting and photometry.

WRIGHT, C. H., 1909, Longevity of fern spores. Kew Bull., p, 427. (cit. HARTT).

WUIST, E. D., 1910, The physiological conditions for the development of monoecious prothallium in *Onoclea struthiopteris*. Bot. Gaz., Vol. 49, pp. 216–219.

CONTENTS.

TABLE I.

Comparison of culture media, Experiment 1.

Material: *Dryopteris viridescens*, lot no. 5, 73 days after collection.
        Woodwardia orientalis, lot no. 5, 73   ,,   ,,   ,,
        Matteuccia struthiopteris, lot no. 5, 84 days after collection.

Culture media: I. 0.1% KNOP's solution.
           II. The same added with 1% agar-agar.
          III. Aqua redestillata.*

Light condition: Diffused light from the north window.

Temperature: 17.5°–21.5°

Species			*Dryopteris*			*Woodwardia*			*Matteuccia*		
Culture media			I	II	III	I	II	III	I	II	III
Per cent of germination	in 2 days		28	0	0	0	0	0	0	0	0
	,, 3 ,,		63	41	0	0	0	0	0	0	0
	,, 5 ,,		94	91	0	43	43	0	17	14	0
	,, 6 ,,		99	96	0	77	72	0	41	26	0
	,, 60 ,,**		—	—	0	—	—	0	—	—	0

    * Inside of the germinator vessels paraffined.

    ** Counting was not practicable for I and II because of the excessive covering area of grown prothallia.

TABLE II.

Comparison of culture media, Experiment 2.

Material: *Osmunda japonica*, lot no. 5, 3 days after collection.
        Osmunda cinnamomea, lot no. 5, 3 days after collection.

Culture media I, II, and III, the same as in Exp. 1.

Light condition: Diffused light from the north window.

Temperature: 18.5°–23°C.

Species			*Osmunda japonica*			*Osmunda cinnamomea*		
Culture media			I	II	III	I	II	III
Per cent of germination	in 1 day		0	0	0	0	0	0
	,, 2 days		47	0	0	0	0	0
	,, 3 ,,		95	65	2	45	30	0
	,, 5 ,,		100	98	6	95	95	0
	,, 7 ,,		100	100	12	98	98	2

TABLE III.

Effect of temperature, Experiment 1.
Equisetum arvense, no. 6, a-d.

Material		no. 6 a		no. 6 b			no. 6 c		no. 6 d
Age of the spore (in days)		5		4			4		5
Temperature		20° : 25°		13°–17°: 25° : 30°			30° : 35°		40°
Per cent of germination	in 1 day	0	15	0	4	6	0	0	0
	„ 2 days	8	44	1	45	50	0	0	0
	„ 3 „	41	81	8	93	73	9	1	0
	„ 4 „	60	86	—	—	—	22	5	0
	„ 5 „	74	90	88	95	86	47	10	0
	„ 6 „	87	92	90	97	91	61	21	0
	„ 7 „	91	94	90	97	94	65	24	0

TABLE IV.

Effect of temperature, Experiment 2.
Osmunda japonica, lot no. 6 a.

Age of the spore (in days)		258			18		8		28		48	
Temperature		6°–10° : 15° : 20°			20° : 25°		25° : 30°		30° : 35°		30° : 40°	
Per cent of germination	in 1 day	0	0	0	0	0	0	3	0	0	0	0
	„ 2 days	0	0	0	0	20	79	86	21	0	5	0
	„ 3 „	0	0	2	3	39	90	93	25	11	28	0
	„ 4 „	0	0	13	12	77	97	92	67	12	48	0
	„ 5 „	0	0	26	34	87	97	95	69	12	48	0
	„ 6 „	0	3.5	31	70	95	97	95	74	20	49	0
	„ 7 „	0	6.5	31	82	95	96	95	80	27	52	0
	„ 8 „		—	—					81	27		
	„ 10 „	0	24	40								

TABLE V.

Effect of temperature, Experiment 3.
Osmunda cinnamomea, lot no. 6.

Age of the spore (in days)		254			24		11		35		45	
	Temperature	6°–10°	15°	20°	20°	25°	25°	30°	30°	35°	30°	40°
Per cent of germination	in 1 day	0	0	0	0	0	0	0	0	0	0	0
	„ 2 days	0	0	0	6	15	51	27	28	6	9	0
	„ 3 „	0	0	8	12	35	60	50	65	16	36	0
	„ 4 „	0	0	29	58	67	85	66	71	32	49	0
	„ 5 „	0	0	45	70	77	87	84	75	38	68	0
	„ 6 „	0	0.5	53	76	83	90	84	76	43	70	0
	„ 7 „	0	4	68	79	85	90	90	80	69	75	0
	„ 10 „	0	21	80								

TABLE VI.

Effect of temperature, Experiment 4.
Dryopteris viridescens, lot no. 8.
Age of the spore, 6 days after collection.

	Temperature	10°–11°	10°–17°	20°	25°	30°	35°	40°
Per cent of germination	in 1 day	0	0	2	10	0	0	0
	„ 2 days	0	0	31	71	11	12	0
	„ 3 „	0	39	61	93	24	21	0
	„ 4 „	0	67	84	97	36	35	0
	„ 5 „	0	83	92	99	46	43	0
	„ 6 „	4	88	96	99	52	48	0
	„ 7 „	14	90	98	100	54	49	0

TABLE VII.

Effect of temperature, Experiment 5.
Woodwardia orientalis, lot no. 8.
Age of the spore, 6 days after collection.

	Temperature	10°–11°	10°–17°	20°	25°	30°	35°	40°
Per cent of germination	in 1 day	0	0	0	0	0	0	0
	„ 2 days	0	0	0	0	4	0	0
	„ 3 „	0	0	4	43	59	10	0
	„ 4 „	0	0	40	87	78	37	0
	„ 5 „	0	0	81	88	86	70	0
	„ 6 „	0	15	88	90	91	86	0
	„ 7 „	0	55	90	92	94	90	0

166 Y. OKADA

TABLE VIII.

Effect of illumination, Experiment 1.
Equisetum arvense, lot no. 6 c.
Age of the spore, 23 days after collection.
Light source, gas-filled tungsten-filament lamp except for the last column.

Temperature	Room temp. (average of daily maxima, 17°; minima, 15°)					25°
Illumination (in m c.)	1250	625	313	156	78	312 (vacuum tungsten filament lamp)
Per cent of germination in 3 days	57	52	20	10	0	—
„ 7 „	59	58	23	24	8	94
„ 14 „	58	71	74	27	8	—

TABLE IX.

Effect of illumination, Experiment 2.
Osmunda japonica, lot no. 6 a.
Age of the spore, 16 days after the collection.
Light source, gas-filled tungsten lamp except for the last 3 columns.

Temperature	Room temp. (average of daily maxima, 17°; minima, 15°)					Sunlight direct	Do. diffused	25°
Illumination (in m c.)	1250	625	313	156	78			312 (vacuum tungsten lamp.)
Per cent of germination in 3 days	51	19	9	2	0	5	54	—
„ 7 „	86	85	71	69	57	—	91	98
„ 14 „	96	96	98	95	91	—	96	—

TABLE X.

Effect of illumination, Experiment 3.
Osmunda cinnamomea, lot no. 6.
Age of the spore, 11 days after collection.
Light source, gas-filled tungsten lamp except for the last three columns.

Temperature	Room temp. (average of daily maxima, 22°; minima, 19°)					Sunlight direct	Do. diffused	25°
Illumination (in m c.)	1250	625	313	156	78			312 (vacuum tungsten lamp.)
Per cent of germination in 3 days	87	81	—	6	0	0	66	—
„ 7 „	88	89	—	54	2	0	91	97
„ 14 „	89	90	—	82	83	0	98	—

TABLE XI.

Effect of illumination, Experiment 4.
Dryopteris viridescens, lot no. 6.
Age of the spore, 54 days after collection.
Light source, gas-filled tungsten lamp except for the last 3 columns.

Temperature		Room temp.					(average of daily maxima, 17° minima, 15°)		25°
Illumination (in m c.)		1250	625	313	156	78	Sunlight direct	Do. diffused	312 (vacuum tungsten lamp.)
Per cent of germination	in 3 days	88	81	54	44	18	—	—	— ·
	„ 7 „	98	97	93	91	92	· –	—	98
	„ 14 „	98	96	95	92	93	—	—	—

TABLE XII.

Per cent of germination in weak light.

Species	Age of spore after collection	Temperature*	Per cent of germination	Illumination		
				5 m c.	1.25 m c.	in diffused sunlight
Equisetum arvense lot no. 6 d	4 days	18°–15°	in 7 days	21%	21%	— ·
			„ 14 „	36%	38%	—
Dryopteris viridescens lot no. 6	40 „	18°–15°	„ 7 „	23%	3%	—
			„ 14 „	54%	4%	—
Osmunda japonica lot no. 6 b	11 „	21°–19°	„ 7 „	8%	2%	92%
			„ 14 „	17.5%	2.5%	94%
Osmunda cinnamomea lot no. 6	11 „	19°–13°	„ 7 „	1%	0%	83%
			„ 14 „	3%	0%	84%

* Average of the daily maxima and minima.

TABLE XIII

Germination test in the dark, Experiment 1.
Equisetum arvense, lot no. 6 d.
Age of the spore, 2 days after collection.

	Temperature condition	Per cent of germination in 10 days	Do. in 7 days of subsequent ex- posure to diffused sunlight
In the dark	30° thermostat	0	0
	25° thermostat	0	0
	Laboratory room (19°–15°)	20	62
In diffused sunlight	Laboratory room (19°–15°)	98	—

TABLE XIV.

Germination test in the dark, Experiment 2.
Osmunda japonica, lot no. 6 b.
Age of the spore, 11 days after collection.

	Temperature condition	Per cent of germination in 10 days	Do. in 7 days of subsequent ex- posure to diffused sunlight
In the dark	30° thermostat	0	65
	25° thermostat	8	82
	Laboratory room (21°–19°)	0	89
	Glass house (29°–15°)	0	94
Instantaneous exposure to the mid-day sunlight, 1 minute daily	Glass house (26°–15°)	11	95
Do. 10 minutes daily	,,	24	95
In diffused sunlight	Laboratory room (21°–19°)	94	—

TABLE XV.

Germination test in the dark, Experiment 3.
Osmunda cinnamomea, lot no. 6.
Age of the spore, 11 days after collection.

	Temperature condition	Per cent of germination in 10 days	Do. in 7 days of subsequent exposure to diffused sunlight
In the dark	30° thermostat	0	0
	25° thermostat	0	75
	Laboratory room (19°–13°)	0	80
	Glass house (33°–14°)	0	61
Instantaneous exposure to the mid-day sunlight, 1 minute daily	Glass house (33°–14°)	0	66
Do. 10 minutes daily	,,	0	73
In diffused sunlight	Laboratory room (19°–13°)	85	—

TABLE XVI.

Germination test in the dark, Experiment 4.
Dryopteris viridescens, lot no. 6.
Age of the spore, 38 days after collection.

	Temperature condition	Per cent of germination in 10 days	Do. in 7 days of subsequent exposure to diffused sunlight
In the dark	30° thermostat	0	0
	25° thermostat	0	74
	Laboratory room (19°–15°)	0	82
Instantaneous exposure, 1 minute daily at noon to diffused sunlight	Laboratory room (19°–15°)	13	86
In diffused sunlight	Laboratory room (19°–15°)	96	—

Table XVII.

Germination test in the dark, Experiment 5.
Dryopteris viridescens, lot no. 8.
Age of the spore, 17 days after collection.
Temperature, 25° thermostat.

	Per cent of germination in 7 days	Do. in 5 days of subsequent exposure to 312 m c. vacuum tungsten lamp
In the dark	0	99
312 m c. vacuum tungsten lamp	99	—

Table XVIII.

Germination test in the dark, Experiment 6.
Woodwardia orientalis, lot no. 8.
Age of the spore, 17 days after collection.
Temperature, 25° thermostat.

	Per cent of germination in 7 days	Do. in 5 days of subsequent exposure to 312 m c. vacuum tungsten lamp
In the dark	0	93
312 m c. vacuum tungsten lamp	91	—

Table XIX.

Germination test under anaerobic condition.

Species; Age of the spore	Temperature	Illumination	Per cent of germination		
			in 7 days under anaerobic condition	in 7 days since the subsequent exposure to aerobic condition	Control culture, in 7 days in aerobic condition from the beginning
Equisetum lot no. 6 d 2 days	19°–15°	Diffused light	0	0	98

Species; Age of the spore	Temperature	Illumination	Per cent of germination		
			in 7 days under anaerobic condition	in 7 days since the subsequent exposure to aerobic condition	Control culture, in 7 days in aerobic condition from the beginning
Dryopteris lot no. 6 38 days	19°–15°	Diffused light	0	0	96
Dryopteris lot no. 8 20 days	25°	312 m c. vacuum tungsten lamp	0	0	99
Woodwardia lot no. 8 20 days	,,	,,	0	87	88
Osm. japonica lot no. 6 b 11 days	21°–19°	Diffused light	0	65	92
Osm. cinnamomea lot no. 6 11 days	,,	,,	0	32	84

TABLE XX.

Comparison of the life of the spore.
1. *Woodwardia orientalis,* lot no. 5.

Age of the spore (in days)		68	75	82	92	107	127	138	174	191	244
Per cent of germination	10 days	79	84	85	78	77	58	21	0	0	0
	21 days	—	—	—	—	—	—	—	1	0	0

2. *Matteuccia struthiopteris,* lot no. 5.

Age of the spore (in days)		79	86	93	103	118	138	149	185	202
Per cent of germination	10 days	58	56	72	58	53	8	0	0	0
	21 days	—	—	—	—	—	—	—	—	—

3. *Dryopteris viridescens*, lot no. 5.

Age of the spore (in days)		68	75	82	92	107	127
Per cent of germination	10 days	96	98	67	52	0	0
	21 days	-	—	—	—	0	0

4. *Osmunda cinnamomea*, lot no. 5.

Age of the spore (in days)		23	43	54
Per cent of germination	7 days	68	7	0
	14 days	82	10	0

5. *Osmunda japonica*, lot no. 5

Age of the spore (in days)		23	43	54
Per cent of germination	7 days	5	0	0
	14 days	8	0	0

6 *Equisetum arvense*, lot no. 5.

Age of the spore (in days)	10	24
Per cent of germination in 7 days	87	0

TABLE XXI.

Effect of different storage, Experiment 1.
Equisetum arvense, lot no. 7.
Age of the spore, i. e. the duration of the storage is expressed in days (Tables XXI–XXIV).

	Storage condition*	Age of the spore	4	10	13	19	23	29	33	36	39	52	59
Per cent of germination in 7 days	I		97	70	12	4	9	8	1	3	1	0	0
	II		98	79	41	79	33	31	9	3	2	3	0
	III		95	21	26	4	4	0	0				
	IV		99	54	28	6	0	0					
	V		98	9	8	6	2	0.5	0	0			
	VI		98	23	75	6	12	2	0	0			

Per cent of germination in 7 days	Storage condition* / Age of the spore	4	10	13	19	23	29	33	36	39	52	59
	VII	97	0	0								
	VIII	98	85	78	0	0						
	IX	97	64	0	0							
	X	96	61	20	0	0						
	XII	97	—	—	—	—	—	—	—	—	11	-

In these series of experiments (Tables XXI–XXIV), the following sets of combination of factors were studied which are tabulated below:

	Temperature-relation	Light-relation	Moisture-relation
I	refrigerator	dark	dry
II	,,	,,	non-dry
III	25° thermostat	,,	dry
IV	,,	,,	non-dry
V	room temperature	,,	dry
VI	,,	,,	non-dry
VII	,,	diffused light	dry
VIII	,,	,,	non-dry
IX		red light	,,
X		blue light	,,
XI	(–16°)–(–11°)	dark	dry
XII	,,	,,	non-dry
XIII	room température	diffused light	,, in H_2-gas

TABLE XXII.

Effect of different storage. Experiment 2.
Osmunda japonica, lot no. 6.

Storage condition	Age of the spore / Per cent germ.	16	40	90	124	152	185	207	340	388	422	600	656
I	in 7 days	81	50	—	39	13	16	10	—	13	1	0	0
	,, 14 ,,	86	63	34	39	16	24	15	21	16	1	0	0

Storage condition	Age of the spore / Per cent germ.	16	40	90	124	152	185	207	340	388	422	600	656	895
II	in 7 days	62	54	—	0	0								
	,, 14 ,,	72	72	0	0	0								
III	,, 7 ,,	79	53	—	31	21	11	7	7	0	0			
	,, 14 ,,	79	55	15	31	11	13	13	4	0	0			
IV	,, 7 ,,	4	0	—										
	,, 14 ,,	4	0	0										
V	,, 7 ,,	54	51	—	8	0	0							
	,, 14 ,,	54	50	14	6	0	0							
VI	,, 7 ,,	56	8	—	0									
	,, 14 ,,	60	9	0	0									
VII	,, 7 ,,	34	7	—	0									
	,, 14 ,,	37	6	0	0									
VIII	,, 7 ,,	64	0	—										
	,, 14 ,,	70	0	0										
IX	,, 7 ,,	52	0	0										
	,, 14 ,,	60	0	0										
X	,, 7 ,,	38	9	0										
	,, 14 ,,	42	0	0										
XII	,, 7 ,,	—	—	—	52	53	59	46	40	45	19	40	31	24
	,, 14 ,,	—	—	—	69	68	60	42	40	47	21	42	30	29
XIII	,, 7 ,,	71	20	accidentally lost.										
	,, 14 ,,	78	18											

TABLE XXIII.

Effect of different storage, Experiment 3.
Osmunda cinnamomea, lot no. 6.

Storage condition	Age of the spore / Per cent germ.	28	78	112	140	173	195	328	376	410	488	564	882
I	in 7 days	72	—	55	29	39	24	35	33	—	3	1	0
I	„ 14 „	74	40	62	38	40	24	35	32	—	3	3	0
II	„ 7 „	76	—	9	0								
II	„ 14 „	81	48	14	0								
III	„ 7 „	63	—	54	34	30	14	16	2	0	0		
III	„ 14 „	71	54	57	34	30	24	17	3	0	0		
IV	„ 7 „	0	—										
IV	„ 14 „	0	0										
V	„ 7 „	52	—	14	9	7	—	11	3	0	0		
V	„ 14 „	67	27	11	10	9	10	12	7	1	0		
VI	„ 7 „	60	—	0									
VI	„ 14 „	60	0	0									
VII	„ 7 „	27	—	0									
VII	„ 14 „	26	0	0									
VIII	„ 7 „	30	—	0									
VIII	„ 14 „	29	0	0									
IX	„ 7 „	27	—	0									
IX	„ 14 „	32	0	0									
X	„ 7 „	27	—	0									
X	„ 14 „	32	0	0									
XII	„ 7 „	—	—	58	62	53	37	48	55	—	49	49	46
XII	„ 14 „	—	—	67	62	58	44	43	60	—	51	50	51
XIII	„ 7 „	45	—	0									
XIII	„ 14 „	62	2	0									

Y. OKADA

TABLE XXIV.

Effect of different storage, Experiment 4.
Dryopteris viridescens, lot no. 6.

Storage condition	Age of the spore / Per cent germ.	34	65	89	139	173	201	234	256	293	317
I	in 7 days	84	54	81	—	69	12	0	0	0	0
	,, 14 ,,	100	79	97	86	77	43	6	1	0	0
II	,, 7 ,,	98	100	94	—	0	0				
	,, 14 ,,	98	98	99	92	0	0				
III	,, 7 ,,	64	2	0	—						
	,, 14 ,,	97	10	0	0						
IV	,, 7 ,,	70	0	0							
	,, 14 ,,	76	0	0							
V	,, 7 ,,	73	47	40	—	0					
	,, 14 ,,	98	85	26	0	0					
VI	,, 7 ,,	79	11	0	—						
	,, 14 ,,	97	21	0	0						
VII	,, 7 ,,	71	13	0	· -						
	,, 14 ,,	87	17	0	0						
VIII	,, 7 ,,	83	97	0	---						
	,, 14 ,,	95	97	0	0						
IX	,, 7 ,,	4	0	0							
	,, 14 ,,	20	0	0							
X	,, 7 ,,	40	0	0							
	,, 14 ,,	69	0	0							
XIII	,, 7 ,,	83	22	0	—						
	,, 14 ,,	91	51	0	0						

TABLE XXV.

Water content of the spores.

Material	Water content (in per cent fresh weight)		
Woodwardia orientalis, lot no. 8.			5.9
Dryopteris viridescens, lot no. 6.	9.80		
,, no. 8.	13.72	average	11.76
Osmunda cinnamomea, lot no. 6.	16.58		
,, no. 7.	16.25	,,	16.42
Osmunda japonica, lot no. 6 a.	17.51		
,, no. 7.	16.99	,,	17.25
Equisetum arvense, lot no. 6 c.	45.50		
,, no. 7	52.76	,,	49.18

TABLE XXVI.

Respiration intensity of the spore.

Species	Lot	Age of the spore (in days)	CO$_2$ produced in mg. per 24 hours per 1 g. of the material	
			at 25°	at 15°
Equisetum arvense	no. 7	6	18.7	--
,,	,,	25	13.53	6.01
,,	,,	33	10.23	--
Woodwardia orientalis	no. 8	10	0.57	0.03
Dryopteris viridescens	,,	,,	0.11	0
Osmunda cinnamomea	no. 7	5	0.33	0.26
Osmunda japonica	,,	6	0.33	0

Table XXVII.

Specific difference in the catalase content.

Species	Equisetum arvense lot no. 7.			Osmunda japonica lot no. 7.			Osmunda cinnam. lot no. 7.			Dryopteris viridescens lot no. 8.			Woodwardia orientalis lot no. 8.		
Age of the spore	5 days			5 days			5 days			13 days			13 days		
Dry matter in per cent fresh weight	47.24			83.01			83.75			86.28			94.10		
Time in minute	5	10	20	5	10	20	5	10	20	5	10	20	5	10	20
O_2 evolved in cc. per 0.1 g. fresh weight	58	76	82	118	164	189	150	182	195	74.3	93	100	33	43	50.3
O_2 evolved in cc. per 0.1 g. dry matter	107	140	151	142	198	228	179	217	232	86.1	108	116	35.1	45.7	53.5
Per cent of germination in 17 days	98			98			99			100			91		

Table XXVIII.

Catalase activity and germination capability as related to the age of the spore, Experiment 1.
Equisetum arvense, lot no. 7.

Age of the spore	5 days			13 days			26 days			1 year*		
Dry matter in per cent fresh weight	47.24			49.0			50.4			92.0		
Time in minute	5	10	20	5	10	20	5	10	20	5	10	20
O_2 evolved in cc. per 0.1 g. fresh weight	58	76	82	24.3	36	45.3	17	24	27.5	6.7	9.9	11.8
O_2 evolved in cc. per 0.1 g. dry matter	107	140	151	57.4	85	107	38	54	62.1	7.3	10.8	12.8
Per cent of germination in 7 days	98			41			31			0		

*In this case, spores kept in dry condition was used as the non-dried material was moulded.

Table XXIX.

Catalase activity and germination capability as related to the age of the spore, Experiment 2.

Osmunda japonica, lot no. 7.

Age of the spore		5 days			53 days			149 days			1 year		
Dry matter in per cent fresh weight		83.01			91.66			92.00			92.3		
Time in minute		5	10	20	5	10	20	5	10	20	5	10	20
O₂ evolved in cc.	per 0.1 g. fresh weight	118	164	189	115	149	168	51	76	88	41	54	58
	per 0.1 g. dry matter	142	198	228	126	163	183	55	83	96	44	59	63
Per cent of germination	in 7 days	95			67			38			13		
	„ 14 „	98			69			40			16		

Table XXX.

Catalase activity and germination capability as related to the age of the spore, Experiment 3.

Osmunda cinnamomea, lot no. 7.

Age of the spore		5 days			48 days			141 days			1 year		
Dry matter in per cent fresh weight		83.75			92.24			92.68			93.0		
Time in minute		5	10	20	5	10	20	5	10	20	5	10	20
O₂ evolved in cc.	per 0.1 g. fresh weight	150	182	195	76	103	114	67	92	103	63	92	100
	per 0.1 g. dry matter	179	217	232	82	112	124	72	99	111	68	99	108
Per cent of germination	in 7 days	99			70			55			32		
	„ 14 „	99			76			55			33		

Table XXXI.

Effect of temperature and moisture condition in the storage method on the catalase content, Experiment 1.

Equisetum arvense, lot no. 7.

Age of the spore (duration of the storage), 35 days.

Temperature condition		In the refrigerator						In 25° thermostat					
Moisture condition		Dry			Not dry			Dry			Not dry		
Time in minute		3	5	10	3	5	10	3	5	10	3	5	10
O₂ evolved in cc.	per 0.1 g. fresh weight	62.5	83	99.5	9.5	15	24	34.8	49.8	59.3	64.5	88.5	112
	per 0.1 g. dry matter	68.5	91	109	18.5	29.1	46.6	39.4	56	67.1	—	-	—
Per cent of germination in 7 days		1			9			0			0		

Table XXXII.

Effect of temperature and moisture condition in the storage method on the catalase content, Experiment 2.

Osmunda japonica, lot no. 7.

Age of the spore (duration of the storage), 53 days.

Temperature condition		In the refrigerator					
Moisture condition		Dry			Not dry		
Time in minute		5	10	20	5	10	20
O₂ evolved in cc.	per 0.1 g. fr. wt.	115	149	168	41	63	77
	per 0.1 g. dry wt.	126	163	183	50	76	93
Per cent of germination	in 10 days	67			0		
	„ 17 „	69			0		

TABLE XXXIII.

Effect of temperature and moisture condition in the storage method on the catalase content, Experiment 3.
Osmunda cinnamomea, lot no. 7.
Age of the spore (duration of the storage), 48 days, 141 days.

Temperature condition		In the refrigerator (48 days)						In 25° thermostat (48 days)						In the refrigerator 141 days			In 25° thermostat (141 days)		
Moisture condition		Dry			Not dry			Dry			Not dry			Dry			Dry		
Time in minute		5	10	20	5	10	20	5	10	20	5	10	20	5	10	20	5	10	20
O_2 evolved in cc.	per 0.1 g. fr. wt.	76	103	114	55	77	95	122	162	176	54	70	86	67	92	103	81	109	123
	per 0.1 g. dry wt.	82	112	124	69	96	119	159	211	230	65	85	104	72	99	111	106	142	160
Per cent of germination	in 10 days	70			38			70			0			55			12		
	„ 17 „	76			43			70			0			55			16		

TABLE XXXIV.

O_2 evolution as influenced by the different procedure, Experiment 1.
Equisetum arvense, lot no. 7.

Age of the spore		5 days			13 days			1 year		
Time in minute		5	10	20	5	10	20	5	10	20
O_2 evolved (in cc.) per 0.1 g. of the material	spores ground	58	76	82	24.3	36	45.3	6.7	9.9	11.8
	„ intact	8.2	17.4	42.4	4.5	18.3	24.0	6.7	9.2	11.0

TABLE XXXV.

Do., Experiment 2.
Osmunda japonica, lot nos. 6, 7.
Spores kept dry in the refrigerator.

Origin & age of the spore		no. 7, 5 days			no. 6, 1 year		
Time in minute		5	10	20	5	10	20
O_2 evolved (in cc.) per 0.1 g. of the material	spores ground	118	164	189	81	114	132
	„ intact	10.8	19.0	34.0	10.1	21.5	51.1

TABLE XXXVI.

Do., Experiment 3.
Osmunda cinnamomea, lot nos. 6, 7.
Spores kept dry in the refrigerator.

Origin & age of the spore		no. 7, 5 days			no. 6, 1 year		
Time in minute		5	10	20	5	10	20
O₂ evolved (in cc.) per 0.1 g. of the material	spores ground	150	182	195	115	168	186
	„ intact	5.8	14.0	28.0	4.5	10.3	28.8

P. S. After the manuscript was finished, I found a paper in which the relation of the longevity of pollen grains to the humidity in the storage condition is dealt: HOLMAN, R. M. and F. BRUBAKER, 1926, On the Longevity of Pollen. Univ. of Calif. Publ. in Bot., Vol. 13, No. 10. It is shown in the paper (pp. 183–184) that the vitality of pollen grains is kept better in artificially desiccated conditions than in ordinary air dry condition (i. e., exposed to the laboratory atmosphere), for the most species studied. It seems, therefore, a common property is shared in this respect by the pollen grains and the fern spores as a general rule (c. f. p. 151).

The Seasonal Variation of the Glycogen Content in the Oyster, *Ostrea circumpicta* Pils.*

By

Katsumi Okazaki† and Satarô Kobayashi.
(The Biological Institute, the Tôhoku Imperial University, Sendai.)

Introduction.

Bizio (1866) found 9.5% of glycogen in the shell-freed total mass of the oyster, *Ostea edulis.*

Mitchell (1915) reported the presence of the seasonal variation of glycogen in the cultured oysters at several districts in Massachusetts, in the United States. Using 12–20 individuals in each determination, he found that the glycogen content showed the minimum early in July, then it steadily increased in summer, though a slight fall occurred before the onset of the cold weather. The glycogen content reached the maximum of 20% (in dried material) in winter.

Russell (1923) studied also the seasonal variation in regard to the various chemical constituents of the cultured oysters in England. Each determination was made on the sample of 50 oysters. He showed among others that the glycogen content gradually declined from January to June, when it reached the minimum (about 20% in the dry weight). After June up to September, it rose rapidly but from September to December, the rate of increase became slower.

The glycogen content in the adductor muscle of Monomyaria has been determined by several investigators, but the seasonal variation was observed only by few. 2–2.4% of glycogen was found in the adductor muscls of *Pecten irradians* by Chittendon (1875). Boyland (1928) observed the changes of the glycogen content in the large adductor muscle of *Pecten opercularis*, in the different stages of the

reproductive cycles.   BOYLAND found 0.61–1.11% of glycogen between
the 1st and 11th days of January.

So far as we are aware the glycogen content was determined with
the oysters which were grown under special care' in the oyster bed
for commercial purposes, and none was determined in the oysters
grown in the natural state without human care.

The present work was therefore undertaken to determine whether
or not a difference is shown as to the glycogen content between the
oysters grown wild and those grown in the oyster-bed as well as in
the different seasons.

For this purpose the oyster, *Ostrea circumpicta*, which is found in
abundance in Mutsu Bay, was used and the variations according to
the seasons were observed during March, 1928, to February, 1929.
Although it was the intention of the writers to carry out monthly
examination, only a few determinations were made during the season
from November to March, owing to the unfavorable weather in winter.
We may mention that in Mutsu Bay, where *Ostrea circumpicta* is
found, the oyster cultivation was never attempted due probably to
high salinity, rocky bottom and reefs, probable scantiness of the proper
plankton organisms, etc., and thus the oysters which are found there
are grown wild.

We wish to acknowledge gratefully much valuable criticism from
Prof. S. HATAI, and our thanks are also due to the members of the
Marine Biological Station at Asamushi, who helped us with much
kindness during the course of the present work.

MATERIAL AND METHOD.

The oysters employed in this experiment were collected always
from a definite place in Mutsu Bay near the Marine Biological Station.
Glycogen was estimated by PFLÜGER's method. The soft body was
removed from the shell, and was weighed after quickly wiping the
body surface with clean cheese cloth. In order to prevent the loss
of the body fluid, the adductor muscle was dissected out in a large
shallow porcelain dish and it was weighed separate from the remainder.
The samples thus obtained were heated for ten minutes in boiling
water to prevent further chemical alteration of glycogen. The heated

materials were dissolved in 60% solution of potassium hydroxide and subsequent analysis was carried out in the Biological Institute at Sendai.

RESULTS.

The results are shown in Tables 1, 2, and 3, and the graphical representation in Fig. 1.

From the data shown in Tables 2 and 3, of the glycogen content in the whole body without the adductor muscle and that in the muscle itself respectively, one may estimate the content in the whole body if these data are brought together, but we have also made direct determinations on the whole body, fearing that while removing the adductor muscle, the body fluid might be lost unawares.

From Tables 1 and 2, one notices at once that the glycogen content varies widely not only with the seasons but also with the individuals.

As to the variations due to the seasons, we notice from the average that the rise and fall of the glycogen content in the whole body and the body without the adductor muscle closely resembled each other, and a steady increase is shown from September or October on to July, when it reaches the maximum, which is followed by a sudden decrease in August.

The decrease continues till the month of September at which period it becomes minimum (1.1%). The significance of the sudden fall which was noted in April is not clear, but we are rather inclined to believe that it was merely casual, and thus the whole tendency of the curves may be considered as increasing after the month of October. Whether or not such cyclical variation occurs regularly in each year is not yet determined. It seems highly probable that the sudden fall of glycogen in August is related to the spawning as was already pointed out by MITCHELL (1915), since the spawning of the oyster in Mutsu Bay seems to occur about the middle of August to the middle of September, at which period the glycogen content became minimum. Such a great fall of glycogen content was reported by MITCHELL (1915) to occur early in July in which the spawning takes place in the oysters, while according to RUSSELL (1923) this fall of glycogen occurs in June.

Whether the occurrence of the spawning of *Ostrea circumpicta* in a later season than that of the two other species mentioned above be due

to the difference of the species is not clear.   However that an increase
after the month of October in our oyster was due to the restoration
after the spawning cannot be doubted.   The maximum storage of
glycogen, which occurs in August in the English oysters and in Novem-
ber in the American oysters, occurs at about the month of March.

The content of the glycogen in the whole body without the adductor
muscle is always much less than that in the whole body from October
to March but becomes greater from April to August. (Table 1, 2, and
Fig. 1.)   This greater accumulation of glycogen from April to August
is probably associated with the greater development of gonads on one
hand and the storage of potential energy for sexual activity during
the subsequent month on the other.

The glycogen content of the adductor muscle shows the similar
seasonal variation, though its content is much smaller.

It is evident from the curves that the glycogen is chiefly stored
rather in the other parts of the body than in the adductor muscle.

According to AMEMIYA (1928), the breeding season of *Ostrea cir-
cumpicta* in southern Japan begins early in July and lasts until late
in July, being at its height in the middle of the month.   Again,
HAMADA (1929) carried on the fertilization experiment at Tottori from
the 26th of July to the end of August using *Ostrea circumpicta*, in-
dicating that the breeding season starts about the beginning of July.

From these data it is clear that the breeding season of *Ostrea
circumpicta* in Mutsu Bay, which lies in the extreme north of the
mainland of Japan (Honshu), takes place in a much later month.
This fact suggests that the breeding season may be controlled chiefly
by the temperature of the sea water.

Comparing the glycogen content in the oyster in Mutsu Bay with
that of cultured oysters which had been determined by the several
investigators, no remarkable difference is found as will be seen from
Table 4.

TABLE   1.

Percentage of glycogen in the whole body.

Date	Weight of Oysters in g.	Glycogen in g.	Percentage	Mean percentage
March 9	42.0 40.2 43.9 35.9 45.4	2.253 1.770 1.985 1.120 1.225	5.365 4.403 4.294 3.120 2.699	4.000
April 9	49.6 40.6 30.1 46.7 46.0	1.622 1.261 0.788 1.034 0.948	3.090 3.090 2.618 2.214 2.061	2.613
May 8	43.9 49.6 44.0 44.5 36.2	1.831 1.381 1.075 0.973 0.755	4.170 3.924 2.444 2.187 2.087	2.962
June 8 ·	31.0 39.3 44.6 49.5 46.5	1.336 1.603 1.803 1.511 1.349	4.311 4.078 4.043 3.053 2.040	3.505
July 8	49.7 41.0 27.2 49.9 39.1	2.340 1.438 0.927 1.182 0.788	4.708 3.498 3.408 2.370 2.015	3.200
August 7	46.2 50.0 38.0 55.5	0.529 0.453 0.321 0.421	1.147 0.906 0.846 0.760	0.915
September 9	35.0 38.5 51.0 34.0 38.0	0.655 0.466 0.582 0.276 0.258	1.870 1.212 1.142 0.812 0.678	1.142
October 9	41.5 48.3 41.5 42.3 35.4	0.953 0.971 0.833 0.811 0.342	2.318 2.009 2.007 1.918 1.052	1.861
November 9	37.2 34.8 43.0	1.317 1.083 1.095	3.540 3.111 2.547	2.543

Date	Weight of Oysters in g.	Glycogen in g.	Percentage	Mean percentage
November 9	38.0 35.0	0.742 0.549	1.952 1.567	2.543
December 26	41.0 51.6 47.2 55.7 45.8	1.181 1.316 1.031 1.226 0.966	3.107 2.551 2.215 2.200 2.109	2.436
February 10	44.0 37.0 49.1 37.2 71.5	2.006 1.465 1.818 1.094 1.888	4.559 3.958 3.702 2.941 2.640	3.560

TABLE 2.

Percentage of glycogen in the body without the adductor muscle.

Date	Weight of Oysters in g.	Glycogen in g.	Percentage	Mean percentage
March 9	43.6 40.6 38.5	2.322 1.534 1.286	4.328 3.778 3.340	3.815
April 9	29.6 29.7 38.8	0.902 0.833 0.881	3.047 2.806 2.271	2.708
May 8	26.1 24.5 36.8	1.196 0.983 1.446	4.582 3.994 3.930	4.169
June 8	45.8 31.5 20.7	1.492 1.228 0.790	3.954 3.898 3.681	3.844
July 8	36.6 37.5 42.1	2.021 1.660 1.740	5.521 4.181 4.132	4.611
August 7	34.2 24.7 46.8	0.699 0.422 0.490	2.044 1.707 1.406	1.719
September 9	39.0 47.1 44.5	0.678 0.662 0.222	1.614 1.407 0.498	1.173

Date	Weight of Oysters in g.	Glycogen in g.	Percentage	Mean percentage
October 8	29.4 37.1 58.4	0.859 0.598 0.449	2.921 1.611 0.769	1.767
November 9	44.3 33.0 39.1	1.134 0.775 0.742	2.559 2.348 1.897	2.263
December 26	43.8 63.8 60.0	1.237 1.350 1.199	2.824 2.116 2.000	2.313
February 10	62.9 48.0 45.9	1.939 1.372 1.203	3.083 2.858 1.456	2.763

TABLE   3.

Percentage of glycogen in the adductor muscle.

Date	Adductor muscle in g.	Glycogen in g.	Percentage
March 9	19.6	0.304	1.543
April 9	22.6	0.223	0.982
May 8	17.6	0.247	1.406
June 8	18.8	0.235	1.250
July 8	20.3	0.323	1.591
August 7	16.2	0.094	0.538
September 9	18.0	0.152	0.846
October 8	18.4	0.168	0.912
November 9	13.2	0.157	1.189
December 27	21.3	0.220	1.032
February 10	23.3	0.339	1.456

TABLE   4.

Percentage of glycogen in the soft body	Species	Locality	Habitat	Investigator
9.5	*Ostrea edulis*			BIZIO (1866)
20.-35. (in the dry weight)	,, ,,	Whistable bed (England)	Cultured	RUSSELL (1923)
2.77–21.50 (in the dried material)		Massachusetts (United States)	,,	MITCHELL (1915)
4.184 (in Winter)	*Ostrea gigas*	Kanazawa (Kanagawa Ken, Japan)	,,	SEKINE (1920)
0.915–4.000	*Ostrea circumpicta*	Mutsu Bay (Japan)	not cultured	OKAZAKI and KOBAYASHI (1929)
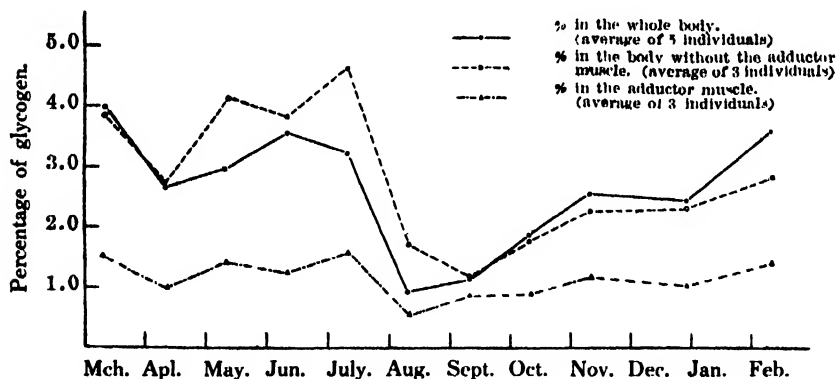
Fig. 1. Showing the seasonal variation of the glycogen content in the oyster, *Ostrea circumpicta* PILS.

SUMMARY.

1. *Ostrea circumpicta* is found naturally in Mutsu Bay.   The glycogen content in *Ostrea circumpita* shows the seasonal variation. It reaches maximum (4% of glycogen) in July and reaches minimum (1%) at about September.   After September it increases continuously

till July again.   Breeding occurs during the months of August and September at which period the glycogen content becomes minimum.

2.   Glycogen content in the adductor muscle is always less than in the rest of the body.

3.   Glycogen content in *Ostrea circumpicta* is not noticeably different from that in the other cultivated oysters.

REFERENCES.

1) AMEMIYA, I. 1928. Ecological studies of Japanese oysters, with special reference to the salinity of their habitats. Jour. of Coll. Agr. Tokyo, Vol. 9, pp. 333-382.

2) BIZIO, M. J. 1866. Sur l'existence du glycogène dans les animaux invertebrés. Compt. Rend. de l'academie des Sci., Tome 62, pp. 675-676.

3) BOYLAND, E. 1928. Chemical changes in muscle. Biochem. Jour., pp, 362-380.

4) CHITTENDON, N. H. 1875. Über Glycogen und Glycocoll in den Muskelgewebe des
   . *Pecten irradians.* Liebig's Ann. der Chem., Bd. 178, S. 266-274.

5) HAMADA, S. 1929. Effect of modification of Vant Hoff solution and sea water upon the fertilization in *Ostrea circumpicta* P. (Japanese). Dobutsugaku Zasshi (A monthly magazine of Zoology), Vol. 40, pp. 515-517.

6) MITCHELL, P. H. 1915. Nutrition of oyster: Glycogen formation and storage. Bull. of the Bureau of Fisheries, Vol. 35, pp. 154-155.

7) ORTON, J. H. 1927. Observations and Experiments on Sex-Change in the European Oyster (*O. edulis.*) Part I. The Change from Female to Male. Jour. Mar. Biol. Assoc., Vol. 14, pp. 1040-1041.

8)* RUSSELL, E. S. 1923. Report on the seasonal variation in the chemical constitution of oysters. Fishery Investigations. London, II. Vol. 1.

9) SEKINE, H. 1920. On the decrease of glycogen in the soaking oyster meats. (Japanese) Suisan Kenkyushi (A monthly Journal on the Fisheries, Fish-culture, Preparation of the marine products and Hydrobiology), Tokyo, Vol. 15, pp. 463-465.

*Indirectly cited through ORTON, J, H.

Lactic Acid and Glycogen in the Adductor Muscles of the Oyster, *Ostrea circumpicta* Pils.*

BY

SATARÔ KOBAYASHI.

(The Biological Institute, the Tôhoku Imperial University, Sendai.)

INTRODUCTION.

The researches on lactic acid in Molluscan muscle have been done by several investigators. HENZE (1905) has found in *Octopus* muscle a small amount of lactic acid (0.01%) and no glycogen, and in 1912 STARKENSTEIN and HENZE were able to detect glycogen in muscles and livers of *Octopus* and *Aplysia*. As to the adductor muscle in Lamellibranchs, BOYLAND (1928) carried on quantitative studies using the adductor muscles in different scallops such as *Pecten opercularis*, *P. maximus*, *P. varius*, etc. On the contents of lactic acid, glycogen, and lower carbohydrates, under various conditions, he concluded that the order of lactic acid production varies as that of activity, and thus the acid production and muscular activity are closely related. Furthermore, the small adductor muscle could produce more lactic acid than the large adductor muscle. FURUSAWA and KERRIDGE (1927) measured the change of pH in the muscles of various marine animals including the adductor muscle of *Pecten opercularis*, and found a definite acid increase (7.06–6.33) on standing for 24 hours after death. The adductor muscle of the oyster consists of two distinct parts, a large rounded and a small white crescent part, the latter beeing attached to the posterior side of the former. Since these two parts are thus anatomically distinct, we may anticipate that they are also functionally different from each other. For convenience, these two parts will be designated, the former " the large adductor muscle " and the latter " the small adductor muscle " in this paper. From this fact just stated, I attempted

*A contribution from the Marine Biological Station, Asamushi, Aomori Ken, and the Biological Institute, Tôhoku Imperial University, Sendai.

to estimate the normal content of lactic acid and its production under experimental conditions in these two adductor mucles in the oyster, *Ostrea circumpicta* PILS, and the results of this investigation are reported in the present paper.

The experiments were undertaken at the Asamushi Marine Biological Station from August to September, 1928, in the spawning season of the oyster, and a few observations on glycogen content of the oysters collected during the months of October and November were added.

<center>TECHNIQUE.</center>

The oysters collected were immediately used, since this investigation requires the materials in the possible freshest condition. From the oysters which without shells weigh 40-80 gms. may be collected 5-7 gms. of the large adductor muscle, and about 1 gm. of the small adductor muscle.

The colorimetric method after MENDEL and GOLDSCHEIDER was used, which requires as small an amount of muscle as 0.3-0.5 gms. for correct estimation of lactic acid content. Immediately after the adductor muscle was removed from the shell, the large and small adductor muscles were quickly separated and were placed on the ice-cooled glass plate. The sample thus collected was minced until it became paste. Then, 0.3-0.5 gms. of each muscle was placed in the previously weighed bottle containing 1 cc. of the 5% metaphosphopric acid solution, which was prepared shortly before the collection of the materials, and the bottle was reweighed in order to find out the weight of the muscle used. A 5% solution of metaphosphoric acid must always be freshly prepared from acidum phosphoricum glaciale just before its use, and in no instance should be it used after 24 hours.

A difinite volume (6-8 cc.) of distilled water was added and stirred with a glass rod. After standing 50-60 minutes, the mixture was shaken and filtered. 2-3 cc. of the filtrates were transferred into a centrifuge tube, and then made up to a difinite volume of 4 cc. by addition of distilled water. To this solution, 1 cc. of saturated copper sulphate solution cooled in the ice water and 1 gm. of calcium hydroxide were mixed, then vigorously shaken and kept standing for 30

minutes. The clear solution was obtained by centrifugalising and was filtered through glass wool or filter paper. The object of this procedure was to remove protein and carbohydrate from the solution. After the removal of protein and carbohydrate by the above procedure, 0.5 cc. of this solution was taken and to this were added 3 cc. of concentrated sulphuric acid (MERCK's reagent, Specific gravity 1.84) in order to produce a concentration of 85% or more of acid. This mixture thus made was warmed in the water bath at the boiling point for 4 minutes. It was then immersed into the ice water for 2 minutes. After cooling, 0.1 cc. of veratrol solution (0.125% in pure alcohol) was added and allowed to stand for 20 minutes.

A pink colour was gradually developed which finally turned into red.

This red coloured solution was compared with the standard solution for indirectly estimating the concentration of lactic acid by means of the DUBOSCQ-PELLIN colorimeter.

The standard solution was prepared by properly mixing the aqueous solution of carbolfuchsin and a small amount of orange G. This solution was previously standardized with the lithium lactate solution of a known concentration (0.0067 gms. in 100 cc. of distilled water).

For the estimation of glycogen in the adductor muscle, the PFLÜGER's method was employed. In this experiment, each adductor muscle of the oyster was cooled in the freezing mixture of sodium chloride and ice before it was used, in order to prevent the conversion of glycogen into lactic acid.

RESULT.

1) LACTIC ACID AND GLYCOGEN CONTENTS OF THE ADDUCTOR MUSCLES IN FRESH MATERIAL.

A) Lactic acid content in the adductor muscles.

The oysters having the body weight of 30–80 gms. without the shell, were treated by the method above described, and the data obtained from 12 individuals are separately given in Table 1. The mean values of lactic acid content of the two kinds of muscle are as follows :

	Extremes	Mean
Large adductor muscle	0.0369–0.0810%	0.0604%
Small adductor muscle	0.0548–0.1319%	0.1012%

TABLE 1.

Lactic acid content in the adductor muscle.

Large adductor muscle			Small adductor muscle		
Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %
0.5503	0.2028	0.0369			
0.4525	0.2031	0.0449	0.3723	0.3070	0.0825
0.3823	0.1736	0.0454			
0.5514	0.2636	0.0478	0.2959	0.2765	0.0934
0.3975	0.2075	0.0522			
0.4049	0.2415	0.0596	0.2806	0.2937	0.1047
0.4390	0.2624	0.0598	0.4585	0.2515	0.0548
0.4530	0.3285	0.0725	0.2861	0.3515	0.1230
0.4980	0.3636	0.0730	0.3263	0.4041	0.1238
0.3768	0.2892	0.0768	0.2930	0.2920	0.1000
0.4637	0.3685	0.0795	0.3957	0.3971	0.1004
0.5151	0.4178	0.0810	0-5973	0.7213	0.1201
			0.3576	0.2965	0.0829
			0.3053	0.2943	0.0964
			0.3483	0.4594	0.1319

As will be seen from Table 1, there are wide ranges of variations in the content of lactic acid among the individuals, and furthermore a greater amount of lactic acid found in the large adductor muscle does not necessarily give a greater amount of the acid in the small adductor muscle. In general, the small adductor muscle contains a slightly greater amount of the acid than the large adductor muscle, in the fresh condition.

B) Glycogen content in the adductor muscles.

MEYERHOF (1920, 1921) has found in the amphibian muscle that the amount of lactic acid formed in muscle is quantitatively related to the amount of glycogen converted. Glycogen in the adductor muscle was determined in the oysters which were collected at the beginning of October and November, 1928.

The results are given in Table 2.

TABLE 2.

Glycogen contents of the adductor muscles.

Date	Number of individuals	Large adductor muscle			Small adductor muscle		
		Weight of muscle in g.	Glycogen in g.	Glycogen, %	Weight of muscle in g.	Glycogen in g.	Glycogen, %
October 9th	10	24.0	0.2679	1.12	9.0	0.1289	1.43
November 10th	11	24.2	0.2586	1.07	11.1	0.1436	1.29

The figures show that a considerable difference is found in the amount of glycogen contained in these two muscles, and the small adductor muscle contains a greater amount of glycogen than the large muscle, as in the case of lactic acid.

The real values of glycogen content as well as lactic acid content in normal condition cannot be obtained, for instance owing to unavoidable stimulation applied to the adductor muscles, when these are removed from the shell. In addition to this disturbance just stated, it is necessary to consider the spawning season in my experiment, since during this period the shell movement becomes more active, as NELSON (1920, 1921) has already observed, and as my material was collected during such a season, it inevitably follows that the glycogen content in muscles in the other seasons may show more or less difference from the values here given.

2) POST-MORTAL PRODUCTION OF LACTIC ACID IN THE
LARGE AND SMALL ADDUCTOR MUSCLES,

FÜRTH (1915) investigated the production of lactic acid during autolysis of normal human muscle and liver compared with those taken

TABLE 3.

Lactic acid production incubated at 45'-48°C.

Body weight in g.	In rigor mortis						In the fresh condition					
	Large adductor muscle			Small adductor muscle			Large adductor muscle			Small adductor muscle		
	Weight of muscle in g.	Lactic acid in mg	Lactic acid %	Weight of muscle in g.	Lactic acid in mg	Lactic acid, %	Weight of muscle in g.	Lactic acid in mg.	Lactic acid %	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %
27.6	0.4560	0.4188	0.0918	0.3250	0.4737	0.1458	0.5514	0.2636	0.0478	0.2959	0.2765	0.0934
33.7	0.3753	0.3496	0.0934	0.3168	0.3932	0.1243	0.3765	0.2892	0.0768	0.2930	0.2920	0.1000
72.5	0.4076	0.4225	0.1037	0.4501	0.6900	0.1533	0.4625	0.2031	0.0449	0.3723	0.3070	0.0625
65.1	0.2706	0.4816	0.1413	0.2706	0.5146	0.1976						
50.0	0.3892	0.5874	0.1510	0.3394	0.5950	0.1753	0.4530	0.3286	0.0725	0.2861	0.3518	0.1230
Mean			0.1165			0.1593			0.0605			0.0997

from a patient who died of diabetes, and found that the latter yield a smaller amount of lactic acid than the normal tissues.

In 1927, SCHMITT-KRAHMER observed the post-mortal production of lactic acid in the several kinds of muscle of pigeon and chick, and reported that the acid production varies with different muscles which are functionally different.

RITCHIE (1926) observed in the muscles of three species of fish, that the amount of lactic acid produced during *rigor mortis* (suspended in phosphate solution) is correlated to the relative activity of the species.

My own observations on the post-mortal production of lactic acid in both adductor muscles in the oyster are now given.

A) *Lactic acid production in the muscles incubated at 45°–48°C.*

The two kinds of muscle freshly collected were separately placed in an open dish, and then incubated for 3 hours in the incubator saturated with moisture to prevent the escape of water from the muscle.

The results of determination are given in Table 3.

As the control, the content of acid was immediately determined on a portion of the same samples, which were incubated.

From these data, the post-mortal production of lactic acid is much greater than that found in the fresh condition, and the small adductor muscle produced a greater amount of acid than the large adductor muscle.

This result thus agrees in general with the results obtained, for instance, by FLETCHER (1907, 1911) who reported that the lactic acid production is gradually increased during incubation at several grades of temperature up to 38° C.

B) *Lactic acid production per hour from each adductor muscle incubated at 37°–38°C.*

Lactic acid was estimated at successive hours after incubation (20 min., 40 min., 1, 1.5, 2.5, 3.5, 6 and 7 hrs. respectively). The results obtained from both the large and small adductor muscles are given separately in Table 4 and 5, and the same data are plotted in Fig. 1.

With the increase in time after incubation both the large and small adductor muscles show steady increase in acid production. I further noticed that the small adductor muscle produces a much greater

TABLE 4.

Lactic acid production of the large adductor muscle incubated in the thermostat (37°-38°C).

Hours after incubation	Body weight in g.								
	50.5			30.0			46.3		
	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %
In fresh material	0.5503	0.2028	0.0369	0.3823	0.1736	0.0454	0.3975	0.2075	0.0522
20 min.	0.3752	0.2116	0.0564				0.3447	0.2356	0.0683
40 min.				0.2830	0.2406	0.0850			
1 hr.	0.3709	0.2520	0.0679				0.3027	0.2290	0.0758
1.5 hrs.				0.4205	0.3508	0.0834			
2.5 hrs.	0.4509	0.3526	0.0782				0.2536	0.2230	0.0879
3.5 hrs.				0.2705	0.2805	0.1054			
6.0 hrs.							0.3691	0.3200	0.0867
6.5 hrs.				0.2555	0.4176	0.1634			
7.0 hrs.	0.5733	0.5990	0.1045						

TABLE 5.

Lactic acid production of the small adductor muscle incubated in the thermostat (37′-38°C).

Hours after incubation	Body weight in g.								
	57.4			50.6			66.8		
	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %
In fresh material	0.3576	0.2965	0.0829	0.3053	0.2943	0.0964	0.3483	0.4594	0.1319
1 hr.				0.3403	0.4566	0.1342			
2 hrs.	0.3451	0.4614	0.1337						
3 hrs.				0.3519	0.7484	0.2127	0.2381	0.4435	0.1863
5 hrs.	0.4180	0.7367	0.1762						
6 hrs.				0.2834	0.7153	0.2524	0.1920	0.5441	0.2834
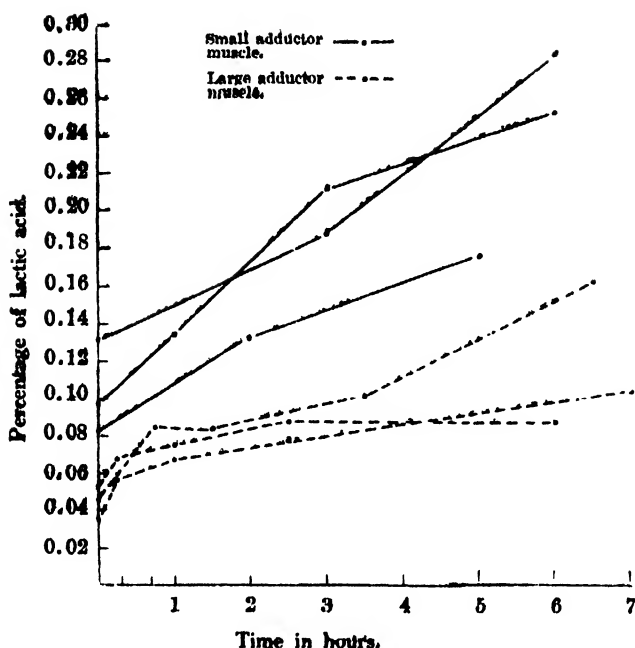
Fig. 1. Showing the post-mortal lactic acid production in the large and small adductor muscles incubated at 37°-38° C.

amount of acid than the large adductor muscle throughout entire period of incubation.

Although there is a strong tendency in the large adductor muscle to relatively greater formation of acid during the earlier period of incubation, such a tendency is not evident in the case of the small adductor muscle.

This difference in the production of acid in each adductor muscle, seems to indicate that these two muscles differ functionally from each other, as BOYLAND (1928) has already pointed out in these two kinds of muscle of *Pecten*.

3) LACTIC ACID CONTENT IN THE ADDUCTOR MUSCLES FATIGUED BY LOADING.

It is generally believed that formation of lactic acid is affected by the amount of work done by the muscle and that a relatively greater

amount is found in fatigued muscle (FLETCHER and HOPKINS 1907, MEYERHOF 1921).

To determine whether or not this statement is also true with the muscles of the oyster the following experiment was made.

2-7 kgms. of weight were hung at the margin of one side of the shells and the other shell was fixed on the support. The oyster thus prepared, was kept in a large vessel which was filled with running sea water.

The shell movement of the oyster was recorded by the kymographic method. The oyster under such circumstance shows vigorous shell movement in rapid succession, but sooner or later its motion stops entirely, owing probably to the fatigue of the muscles concerned. At this period, the adductor muscles were removed from the oyster and the estimation of lactic acid was carried out in the same way as previously stated. The results obtained are given in Table 6.

TABLE 6.

Lactic acid contents in the adductor muscle fatigued by loading.

Body weight in g.	Large adductor muscle			Small adductor muscle			Weight of loading	Time in hours of loading	
	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %	Weight of muscle in g.	Lactic acid in mg.	Lactic acid, %			
32	0.3107	0.2211	0.0712	0.2985	0.3656	0.1225	5	28	Small adductor muscle was cut by loading.
27	0.3800	0.3094	0.0814	0.3158	0.3858	0.1213	2	84	
38	0.4603	0.4277	0.0929	0.4231	0.4744	0.1074	7	60	
25	0.4290	0.4256	0.0985	0.4827	0.4420	0.0916	5	24	
40	0.3279	0.4808	0.1466	0.5274	0.4865	0.0923	7	48	
46	0.3515	0.6319	0.1798	0.3466	0.5930	0.1711	5	24	Small adductor muscle was cut by loading.
Mean			0.1117			0.1361			

The figures given in Table 6 indicate that the lactic acid content of each adductor muscle widely varies among the individuals, but in every instance it is greater than that found in the muscles in normal condition. The mean values of acid content are given in the following:

	fatigued	normal (see page 196)
Large adductor musle	0.1117%	0.0604%
Small adductor muscle	0.1361%	0.1012%

The lactic acid content of the large adductor muscle is slightly less than that of the small adductor muscle, contrasted with much greater difference found in the normal fresh muscles: in other words the acid production is greater in the large adductor muscle than in the small adductor muscle as the result of fatigue.

This difference in the rate of formation of acid seems to mean that the work done by the large adductor muscle is greater, as the result of fatigue, than that done by the small adductor muscle.

From the data shown by the experiment, it may be considered that the small adductor muscle of the oyster *post mortem* can produce a greater amount of lactic acid than the large adductor muscle, as BOYLAND (1928) found in the *Pecten* muscle, but does not so much work as done by the large adductor muscle under loading.

In concluding, I wish to express my sincere thanks to Prof. S. HATAI and the late Assist. Prof. K. OKAZAKI for their valuable suggestions and encouragement during the work.

SUMMARY.

1) Lactic acid was estimated in the large and small adductor muscles of the oyster, *Ostrea circumpicta* PILS, and a few observations on the glycogen content of these two muscles were made of the materials collected during the months of October and November.

2) In the fresh condition, the small adductor muscle contains more lactic acid than the large adductor muscle, and the same relation was also found on glycogen content.

3) The post-mortal production of lactic acid in these two muscles, which were incubated at 45°–48°C. for 3 hours, is much greater than those in the fresh condition, and the small adductor muscle produces a much greater amount of acid than the large adductor muscle.

4) In the post-mortal production of the acid in the successive hours of incubation at 37° 38°C., the small adductor muscle produced at all periods a much greater amount of acid than the large adductor

muscle. A strong tendency of increased acid production is found in
the large adductor muscle at the beginning of incubation, but such
tendency is not evident in the small adductor muscle.

5) In the muscles fatigued by loading 2-7 kgms. of weight, the
lactic acid content of the large adductor muscle is slightly less than
that of the small adductor muscle, though greater in both than in
the normal muscles. The acid production under loading is greater in
the large adductor muscle than in the small adductor muscle.

REFERENCES.

1) BOYLAND, E. 1928. Chemical changes in muscle. Biochem. Journ., Vol. 22, pp
   362–380.
2) FLETCHER, W. M. and HOPKINS, F. G. 1907. Lactic acid in amphibian muscle.
   Journ. of Physiol., Vol. 35, pp. 247–309.
3) FLETCHER, W. M. 1911. On the alleged formation of lactic acid in muscle during
   autolysis and in post-survival periods. Journ. of Physiol., Vol. 43, pp. 286–
   312.
4) FURUSAWA, K. and KERRIDGE, M. T. 1927. The hydrogen ion concentration of
   the muscles of marine animals. Journ. Marin. Biol. Assoc., Vol. 14, pp.
   657–659.
5) FÜRTH, O. 1915. Über die Beziehungen der Milchsäure zum Kohlenhydratstoff-
   wechsel. III. Über die Milchsäurebildung beim menschlichen Diabetes.
   Biochem. Zeitsch., Bd. 69, S. 209–212.
6) HANSEN, A., RIESSER, O. und NAGAYA, T. 1928. Kolorimetrische Bestimmung der
   Milchsäure nach MENDEL und GOLDSCHEIDER. Biochem. Zeitsch., Bd. 196,
   S. 301–308.
7) HENZE, M. 1905. Beiträge zur Muskelchemie der Octopoden. Zeitsch. f. physiol.
   Chem., Bd. 43, S. 477–493.
8) MENDEL, B. und GOLDSCHEIDER, I. 1925. Eine kolorimetrische Mikromethode zur
   quantitativen Bestimmung der Milchsäure im Blut. Biochem. Zeitsch., Bd.
   164, S. 163–174.
9) MEYERHOF, O. 1920. Die Energieumwandlungen im Muskel. III. Kohlenhydrat-
   und Milchsäureumsatz im Froschmuskel. Arch. f. ges. Physiol., Bd. 185, S.
   11 32.
10) MEYERHOF, O. 1921. Die Energieumwandlungen im Muskel. IV. Über die Milch-
    säurebildung in der zerschnittenen Muskulatur. Arch. f. ges. Physiol., Bd.
    188, S. 114–160.
11) NELSON, T. C. 1920. Observations of the feeding habit of oysters. Report. Depart.
    Biol., New Jersey Agricul. Coll. Exper. Stat., New Brunswick, pp. 333–345.
12) NELSON, T. C. 1921. The spawning of Oysters. Report. Depart. Biol., New Jersey
    Agricul. Coll. Exper. Stat., New Brunswick, pp. 296–297.
13) RITCHIE, A. D. 1926. Lactic acid and rigor mortis in fish muscle. Journ. of
    Physiol., Vol. 61, pp. iv–v.

14) RITCHIE, A. D. 1927. Lactic acid in fish muscle and crustacean muscle. British Journ. of exper. Biol., Vol. 4, pp. 327–332.

15) SCHMITT-KRAHMER, C. 1927. Die Milchsäurebildung in roten und weissen und functionell verschiedenen Muskeln der Vögel. Biochem. Zeitsch., Bd. 180, S. 272-287.

16) STARKENSTEIN, E. und HENZE, M. 1912. Über den Nachweis von Glykogen bei Meermollusken (Speziell bei Octopoden und Aplysien). Zeitsch. f. Physiol. Chem., Bd. 82, S. 417-424.

Studies on the pH and the CO_2-Content of the Blood, Pericardial Fluid, and the Body Fluid of the Oyster with Special Reference to their Response to the Altered Condition of Sea Water.

By

SEIJI KOKUBO.

(Marine Biological Station of the Tôhoku Imperial University, Asamushi, Aomori-ken)

INTRODUCTION.

The present investigation has been intended not only to secure the definite data in regard to the hydrogen ion concentration and the CO_2-content in the blood, pericardial fluid, and the body fluid of the oyster, but also to define the relation which exists between the aforementioned fluids and sea water, inasmuch as these fluids stand in close connection to each other in the body of the oyster. Furthermore the sea water which comprises the environmental medium of the animals may be thought to have some direct bearing upon these fluids, and hence the study of this relation must have an important bearing upon the physiology of the oyster.

The saline solution used in the perfusion of the heart of higher animals has the pH closely allied to that of the pH of the blood. Whereas, according to the work of TAKATSUKI[1] which was undertaken in our laboratory, the oyster heart survives better in sea water than in the ordinary saline solution, continuing the pulsation as long as 14 days in some cases, provided the proper temperature is maintained during the experiment. As is well known the composition of the blood of some low marine animals shows a close resemblance to the composition of sea water. I have reported in my previous paper[2] that the pH of the blood of *Arca* (a large bivalve) shows a high alkalinity of pH 7.75 in ordinary cases, which closely resembles that of sea water and makes a distinct contrast with that of the marine fishes which have a much lower pH than this value. I presumed the

long survival of the oyster heart in sea water may be due to the similarity of chemical composition of these solutions, though it was found on the contrary that the blood, pericardial fluid, and body fluid have no similarity to the sea water so far as the value of pH is concerned.

COLLIP[4] found that the coelomic fluid of *Mya arenaria* alters its components, especially its CO_2-content, by exposing the animal to some gas instead of water, and he stated that this change may be attributed to the increase of alkali reserve of coelomic fluid from the dissolution of shell carbonate, on account of an increase of CO_2 in the blood by respiration. Although this is an interesting study, it is to be regretted that the method employed here was relatively rough and also lacked the determination of pH of his ' clam juice '. In my present investigation the pH and CO_2-content of (1) Blood, (2) Pericardial fluid, and (3) Body fluid were determined with a view to find the relation existing between these fluids and the sea water by placing the oyster in different experimental conditions.

I. MATERIAL.

The material employed was a Japanese oyster, *Ostraea circumpicta* PILS. which is found in quantities in the near vicinity of our station.

The heart is situated just above the adductor muscle suspended in the pericardial fluid which fills the pericardial cavity. The pericardial cavity is triangular shaped, and has a length of about 20 mm. in a

specimen of 10 to 15 cm. in shell length. In such a specimen the heart becomes as large as 15 mm. in diameter when fully expanded, filling the whole pericardial cavity.

To obtain the pericardial fluid, a syringe needle was inserted carefully into the cavity so as not to harm the heart and then the fluid was gently drawn out. In a large specimen such as exceeds 15 cm. in length 1.5 cc. of the fluid can be obtained at one time. Though the pericardial cavity becomes empty after this collection. it will be refilled by the fluid after 3-5 minutes. Even when this second accumulated fluid is taken off, the cavity will be filled thrice by the fluid in the course of 30 minutes. Thus some 15 cc. of fluid can be collected from a single oyster by successive collections. The fluid cellected secondly or thirdly is likely to be of the same character as that first taken, and no differences are found in the value of pH when compared with one another. However, the fluid shows an increase of about 0.1–0.2 in pH, one to several hours after the cavity is opened. Such increase of pH which is also seen ' in vitro ' is presumably be attributed to the evaporation of free CO_2 from the fluid.

The blood is collected by inserting the syringe needle into the heart which is suspended in the pericardial fluid. 15 cc. of blood can be collected when a large specimen is used. Although the heart usually shrinks after a blood collection, it swells again after a while and hence 4 to 6 cc. of blood is obtainable, provided the syringe needle remains in the heart. The heart beat continues even after such a quantity of blood is withdrawn, but it does not swell so much as before.

The body fluid is a juice which oozes out from the body tissue when the shell is opened. Strictly speaking, this juice is a mixture of tissue juice, blood, and sea water which remains around the mantle and gills. The body fluid should be collected before other fluids, as it will be abnormally mixed with blood and pericardial fluid when the pericardial cavity is perforated.

To collect these fluids from the specimen the shell was carefully opened so as not to harm the pericardium, and after removing the sea water from the mantle cavity, body fluid was collected in a test tube, and both the blood and pericardial fluid were carefuly collected in the manner already stated, avoiding at the same time contact with

air, and by previously filling a syringe needle with paraffin oil. The fluids thus collected were transferred into the test tube under the paraffin oil.

II. METHOD.

a. Preparation of the breathing water. To change the pH and the CO_2-content of the blood and pericardial fluid ' in vivo ', the oyster was placed for a definite time in sea water in which was added a certain quantity of acid or alkali with a view to decrease or increase the hydrogen ion concentration. The sea water used for experiment was the tap water of the laboratory with the specific gravity of 1.0228 to 1.0245 in varying temperatures and the alkalinity of pH 7.90 to 8.20 in the course of experiment. Two litres of sea water to which an adequate quantity of HCl or NaOH was added was taken in a glass jar measuring about 23 cm. in diamer and 13 cm. in depth, and the oyster was submitted to respiration in this jar. The water was changed during the experiment when needed.

For the acid experiment three gradients of acidity were chosen, that is pH 5.00, pH 3.00, and pH 1.20. For the alkali experiment also, three gradients of pH 9.00, n/100, and n/50 solution of NaOH made with sea water were used. White precipitate of magnessium hydroxide produced by the addition of alkali was removed and the titer was titrated against half normal oxalic acid solution.

b. Determination of pH. The pH of the blood, pericardial, and body fluid was for the most part determined colorimetrically. To the blood placed under the paraffin oil in the test tube was added an indicator and the colour was compared with the standard colour. In normal cases the phenol red was preferred as the indicator, although brom thymol blue or thymol blue was used in cases where the acidity or alkalinity was altered by an addition of acid or alkali. As the blood, pericardial fluid, and body fluid have no considerable turbidity the colorimetrical determination was successfully performed. By this method, however, the results may be accompanied by the salt or protein error, so that the pH was also determined by measuring the E M F by the gas chain method for correcting the errors just mentioned.

The measurement of E M F in blood and other fluids of oyster was anticipated to be very difficult on account of its poor buffer, but contrary to my anticipation the E M F was found to be measured with much ease, as well as with stability and accuracy.

The electrode employed in the present investigation was a micro electrode which was devised by me for the object of measuring the pH of blood (Fig. 1). This electrode has a cylindrical form measuring 5 cm. in length and 0.9 cm. in diameter and makes possible the pH determination by using 1 cc. of the sample. A small piston (a) with platinum electrode slides freely inside the small cylinder (b). In using the electrode the piston (a) is first removed and then the capillary tube (c) is covered by a small rubber cup (d). After pouring the sample into the tube (b) the piston (a) is inserted and the rubber cup (d) is taken off. The piston of the platinum electrode in the tube can be kept anywhere wanted by fixing the sliding piston with a clip (e) attached. For the introduction of hydrogen gas a capillary tube connected with KIPP's apparatus is inserted into the cylinder through the tube (c). When the platinum black is saturated with hydrogen, the piston (a) is pushed slightly, and naturally a

Fig. 1.

small portion of the fluid drops from the tube (c). Herewith the electrode is immersed in the KCl solution by the tube (c), and E M F is measured by a potentiometer in the ordinary way. If, in this case, the electrode is filled with a sample with an indicator, it may be compared with a pH colorimeter and both the electrometric and colorimetric determinations can be simultaneously accomplished. The special advantages of this electrode are first that it needs no agar tube in connecting the sample with the KCl vessel and secondly that the temperature of KCl can be taken as the temperature of the measuring sample, inasmuch as the lower half of the electrode may be dipped into the KCl solution. If this electrode were made smaller, 0.5 or 0.2 cc. of sample would be enough for each determination.
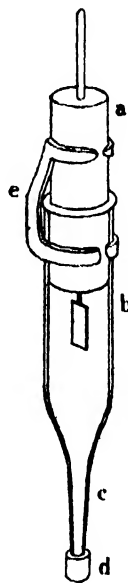
In the present investigation the gas chain arrangement was standar-

dized at least three times a day against standard acetate solution before, during, and after the work. In each determination 7 E M F readings were taken within 30 minutes, and the change of E M F in the range of 1/10 millivolt was carefully observed and averaged.

   c. *Determination of CO_2-content.* The CO_2-content has been determined by a manometric blood gas apparatus of VAN SLYKE. The pipette of our apparatus was of 50 cc. content. Sample used was 1 cc. for each determination, ' S ' i. e. the volume of solution in extracting chamber was 3.5 cc. ' a ' i. e. the volume of gas when the manometer is read at $t°$ was 2.0 cc. The factor ' i ' has been assumed to be 1.017. Therefore vol% factor was taken from VAN SLYKE's article without making any modification. ' C ' correction was determined to be 1.2 mm for an addition of 0.2 cc of 5 n NaOH solution.

III. RESULTS.

A. THE pH OF THE BLOOD, PERICARDIAL FLUID, AND BODY FLUID MEASURED COLORIMETRICALLY.

1. The pH of the Blood.

87 determinations (53 determinations given in table 1, and 34 determinations given in table 5) which were made on the normal individuals showed a pH value of 7.24 in mean. The highest value of all the determinations has been found to be pH 7.80, which was observed in the cases of early winter given in Table 5 (specimen No. 27–34), and the lowest value of all has been found to be pH 6.70 which was observed in the case of No. 16 given in Table 1. All these determinations, however, are considered better divided into the following four groups, inasmuch as they were made out in four different seasons.

Season	pH value	Date of experiment
(1) Early summer	pH 7.15±0.023	VII, 31 VIII, to 4 (Table 1, No. 2–54)
(2) Midsummer	pH 7.33±0.067	VIII, 14 (Table 5, No. 1 -20)
(3) Early autumn	pH 7.18±0.078	X, 2–15 (Table 5, No. 21–26)
(4) Early winter	pH 7.68±0.065	XI, 19 (Table 5, No. 27–34)

From the figures given above one will be struck by the presence

of a strong tendency showing a regular seasonal change, with the exception of early autumn, in which a slight decrease of pH is shown. This decrease in early autumn may possibly be the outcome of a comparatively less number of determinations. At any rate, the rise of the pH in early winter is evident, as can be seen from the regularity of figures secured in that season. Therefore it may be said that the pH of the blood of oyster shows a seasonal variation, decreasing in early summer and increasing in early winter.

Further details regarding the pH of each season will be given in the following.

a. The pH in early summer (Table 1). Fifty three determinations which have been made on the normal individuals in June are enumerated in Table 1. The highest value and the lowest value among these determinations showed pH 7.60 (No. 41) and pH 6.70 (No. 16) respectively, with the value of 7.15 in mean. Accordingly most individuals indicated a weak alkalinity, except in five cases in which the pH values are lower than neutral. Treating these fifty three figures more correctly on the basis of the theory of probablity, the mean error of each determination was found to be ±0.023. The pH of the oyster blood, therefore, may be best represented by pH 7.15 ± 0.023 so far as the present series of determination are concerned.

b. The pH in midsummer (Table 5, No. 1–20). The result of 20 determinations made on the specimens collected in the early half of August are given in Table 5. The highest and the lowest values among these determinations showed pH 7.65 (No. 8, No. 9) and pH 6.80 (No. 7, No. 15) respectively, showing the pH value of 7.33 in mean. The mean error of the mean was ±0.067, showing these figures of somewhat larger magnitude than in the cases of early summer. And in this case, therefore, the pH 7.33 ± 0.067 may be taken as the most correct value fore the midsummer samples.

c. The pH in early autumn (Table 5, No. 21–26). Six determinations have been made on the specimens which were collected in the early half of September. The highest and the lowest values were pH 7.45 and pH 6.90 repectively. The mean value which showed pH 7.18 was lower than that of midsummmer. The mean error of each determination has been found to be ±0.19 and the mean error of the mean to be ±0.078, showing these figures to be the largest

of all the seasons. And hence the pH of the blood of this season becomes pH 7.18 ± 0.078.

  d. The pH in early winter (Table 5, No. 27–34). The pH of the blood in this season has been determined on 8 specimens which were collected on Sept. 19. The highest value in this season showed a pH as high as 7.80, which has never been found in any of the other seasons. The lowest value was by far higher than those of the other seasons, indicating pH 7.30. And in consequence, the mean value was found to show the highest of all the seasons ever determined, giving pH 7.60. The mean errors of each determination and the mean error of the mean have been found to be ± 0.18 and ± 0.065 respectively, showing that these figures are by far smaller than those of the former case. The pH of the blood in this season is best represented by pH 7.68 ± 0.065.

TABLE 1.

No.	Temp.		Specif. grav. of water	pH				Oyster		June 1928	
	Air	Wat.		Water	Blood	Peric. fl.	Body fl.	Leng-th	Bread-th	Date	Hour
1	13.5	13.8	23.00	8.15	—·	6.90	—	13.8	6.5	2	11.00 a m
2	,,	,,	,,	,,	7.20	7.30	—	12.0	7.5	3	11.30 ,,
3	,,	,,	23.10	,,	7.30	7.20	7.30	11.5	8.0	3	11.40 ,,
4	,,	,,	,,	,,	7.20	7.30	—	13.0	8.5	3	11.50 ,,
5	,,	,,	23.10	,,	7.30	7.20	—			3	4.55 p m
6	13.4	13.0	23.40	,,	6.80	6.80	6.75	11.5	7.5	4	4.00 p m
7	,,	,,	,,	,,	7.10	7.20	—	10.0	7.0	4	1.30 ,,
8	,,	,,	,,	,,	7.20	7.20	—	12.0	6.0	4	2.00 ,,
9	,,	,,	,,	,,	7.10	7.10	7.10	11.0	7.0	4	3.00 ,,
10	,,	,,	23.30	,,	7.10	7.10	—	9.5	6.8	5	4.10 ,,
11	13.9	12.7	,,	8.10	7.25	7.30	—	11.0	7.0	5	9.00 a m
12	,,	,,	,,	,,	7.45	7.40	—	15.0	9.3	5	10.30 ,,
13	,,	,,	,,	,,	7.10	7.00	7.00	13.2	10.0	5	11.00 ,,
14	17.5	13.1	,,	,,	7.00	7.00	—	11.0	8.0	5	4.00 p m
15	,,	13.2	,,	,,	7.20	7.20	7.20	12.0	9.0	5	4.30 ,,
16	15.1	14.0	23.60	8.15	6.70	6.70	—	12.5	9.0	6	2.00 ,,
17	,,	,,	,,	,,	7.00	6.90	—	9.5	6.0	6	2.10 ,,
18	,,	,,	,,	,,	7.30	7.20	7.40	13.0	6.5	6	3.00 ,,
19	17.5	14.0	,,	7.90	7.40	7.35	7.40	13.0	10.0	7	9.00 a m
20	18.0	,,	23.50	,,	6.80	6.80	7.10	10.0	8.5	7	9.30 ,,
21	,,	,,	,,	,,	7.30	7.20	7.10	12.0	6.5	7	2.00 p m
22	,,	,,	,,	,,	7.10	7.00	7.00	8.5	7.0	7	2.30 ,,
23	18.0	,,	,,	8.20	7.10	7.10	—	13.0	8.5	7	4.00 ,,
24	,,	,,	,,	,,	7.30	7.20	7.10	9.5	7.0	7	4.30 ,,
25	,,	,,	24.00	,,	7.10	7.10	7.00	11.0	9.0	8	8.00 a m
26	,,	,,	,,	,,	7.20	7.10	7.10	11.0	9.5	8	9.00 ,,
27	19.0	,,	,,	,,	7.30	7.30	7.30	10.0	8.5	8	9.20 ,,

No.	Temp.		Specif. grav. of water	pH				Oyster		June 1928	
	Air	Wat.		Water	Blood	Peric. fl.	Body fl.	Leng-th	Bread-th	Date	Hour
28	19.0	14.0	24.00	8.20	7.20	7.00	6.80	11.0	7.5	8	11.00 a m
29	19.5	,,	,,	8.10	7.10	7.05	7.00	9.0	6.5	8	10.00 ,,
30	21.0	,,	,,	,,	7.30	7.30	7.20	11.0	7.0	8	11.00 ,,
31	,,	16.0	23.10	,,	7.20	7.20	7.10	10.0	7.5	9	1.30 p m
32	,,	,,	,,	,,	7.00	6.90	6.90	10.5	6.0	9	3.00 ,,
33	,,	,,	,,	,,	7.00	6.90	6.90	12.0	10.0	9	4.30 ,,
34	,,	,,	,,	7.90	7.30	7.20	7.20	9.0	4.2	9	4.40 ,,
35	,,	,,	,,	,,	7.00	6.80	6.80	10.5	6.5	9	5.00 ,,
36	20.0	14.5	24.00	,,	7.10	7.05	7.05	13.5	8.0	12	9.00 a m
37	,,	,,	,,	,,	7.30	7.10	7.20	12.5	9.0	12	9.30 ,,
38	20.5	,,	,,	,,	7.10	7.10	7.10	12.0	8.8	12	10.30 ,,
39	21.0	,,	,,	,,	7.25	7.10	7.00	12.0	8.5	12	11.00 ,,
40	,,	,,	,,	,,	7.10	7.00	7.10	10.0	7.5	12	1.00 p m
41	,,	,,	,,	,,	7.60	7.50	7.50	9.0	7.0	12	2.00 ,,
42	19.0	,,	,,	,,	7.30	7.30	7.20	11.0	7.0	12	5.30 ,,
43	,,	,,	,,		7.30	7.30	7.40	9.0	7.0	12	6.00 ,,
44	,,	,,	,,		6.90	6.90	6.90	13.0	9.2	12	6.10 ,,
45	,,	15.5	24.10	8.20	7.10	7.05	7.10	8.5	7.5	13	8.30 a m
46	,,	,,	,,	,,	7.30	7.10	7.30	12.5	7.0	13	8.00 ,,
47	19.5	,,	,,	,,	7.05	7.00	7.00	14.0	7.5	13	10.30 ,,
48	,,	,,	,,	,,	7.10	7.05	7.10	15.0	7.5	13	11.00 ,,
49	,,	,,	,,	,,	7.00	7.10	7.10	11.0	7.6	13	11.20 ,,
50	,,	,,	,,	,,	6.90	6.90	6.90	15.0	9.0	13	11.30 ,,
51	,,	,,	,,	,,	7.20	7.20	7.30	12.0	8.5	13	1.40 ,,
52	,,	,,	,,	,,	7.30	7.20	7.30	12.0	6.5	13	1.45 ,,
53	,,	,,	,,	,,	7.40	7.40	7.40	10.0	8.0	13	2.00 ,,
54	,,	,,	,,	,,	6.80	6.75	7.00	11.0	7.5	13	2.30 ,,

2. The pH of the Pericardial Fluid.

Eighty eight determinations which have been made on the normal individuals showed a pH value of 7.16 in mean. These determinations are now classified into four groups according to the season.

Season	pH	Date of experiment	
(1) Early summer	pH 7.10±0.024	VII, 31 to VIII, 4	(Table 1, No. 1–50)
(2) Midsummer	pH 7.13±0.062	VIII, 14	(Table 5, No. 1–20)
(3) Early autumn	pH 7.12±0.095	IX, 2–15	(Table 5, No. 21–25)
(4) Early winter	pH 7.59±0.076	XI, 19	(Table 5, No. 27–34)

The pH of the pericardial fluid shows a close simility to that of the blood, and consequently similar seasonal changes are also shown, that is, the value of pH increases from early summer towards early winter with the exception of early autumn, where it shows a slight

decrease as in the case of the blood.

    a. **The pH in early summer** (Table 1). According to the 54 determinations which were made on the specimens collected in the early half of June the highest pH of the pericardial fluid is found to be pH 7.50 (No. 41) and the lowest value pH 6.70 (No. 16) with pH 7.10 in mean. Although the pH varied in a range of from pH 7.00 to 7.40 in the majority of cases, 11 individuals showed a faint acid reaction. The mean error of each determination showed ±0.18, a little less than that of the blood, and the mean error of the mean has been found to be ±0.024, showing a little higher value than that of the blood. And hence the pH of pericardial fluid is most correctly represented by pH 7.10±0.024.

    b. **The pH in midsummer** (Table 5, No. 1–20). Among 20 determinations given in the Table 5, we find pH 7.60 in maximum, pH 6.80 in minimum, and pH 7.13 in mean. Naturally the pH value was found to be above the neutral in most cases, with 5 exceptions in which the pH were below neutral, showing a faint alkali reaction. The mean error of each determination showed ±0.28 and the mean error of the mean has been found to be ±0.062, showing somewhat larger magnitudes than those of the cases in early summer. Therefore the pH of the pericardial fluid is most correctly represented by pH 7.13±0.062 in this season.

    c. **The pH in early autumn** (Table 5, No. 21–26). The pH in early autumn was determined on the six individuals which were collected in the early half of September. The highest and the lowest value in this season are found to be the pH 7.40 and pH 6.75 respectively, showing a mean value of pH 7.12. The mean error of each determination showed ±0.23, and the mean error of the mean showed ±0.095, the latter showing the largest value among the four seasons. The pH of the pericardial fluid in this season is represented most correctly by pH 7.12±0.095.

    d. **The pH in early winter** (Table 5, No. 27–34). Eight determinations have been made on the specimen collected in this season. The highest and the lowest values showed pH 7.80 and pH 7.20 respectively. The mean value showed a pH of 7.59 which was the highest mean of all seasons ever determined, and indicated the presence of distinct increase in pH in this season. The mean error of each

determination and the error of the mean showed ± 0.21 and ± 0.076 respectively. Therefore the pH of the pericardial fluid in this season can be regarded to be pH 7.59 ± 0.076.

From what has just been mentioned it follows that the pH of the pericardial fluid of the oyster shows a seasonal change, indicating the highest value in early winter and the lowest value in early summer. And the pH of the pericardial fluid shows a faint alkali reaction even though the alkalinity is a little less than that of the blood.

3. The pH of the Body Fluid.

The pH of the body fluid has been determined on 41 different individuals, and it was found to be 7.50 in maximum, 6.70 in minimum and 7.11 in mean. Among 41 determinations 7 cases showed a pH below 7.00. The mean error of each determination showed ± 0.19 indicating a little larger value than in the case of the blood and pericardial fluid. The mean error of the mean also showed the small value of ± 0.029, and hence the pH of the body fluid becomes 7.11 ± 0.029.

According to the present 41 determinations the pH of the body fluid was found to be a little lower than that of the blood and the pericardial fluid. A comparison of the pH of the three fluids is as follows :

1) Blood   pH 7.21 (mean of all determinations)
2) Pericardial fluid pH 7.16 (ditto   )
3) Body fluid   pH 7.11 (ditto   ).

B. THE pH OF THE BLOOD, PERICARDIAL FLUID, AND THE BODY FLUID MEASURED BY A GAS CHAIN METHOD.

The pH of the blood, pericardial fluid, and the body fluid measured colorimetrically cannot be considered as the true pH value of these fluids because they contain proteins and salts which may produce errors on the colorimetric reading of these fluids. Accordingly the pH of the same samples has been determined both by the gas chain method and the colorimetrical method simultaneously, and the errors were calculated from these results.

The blood and pericardial fluid of the oyster tend to change their pH during the experimental treatment by the loss of CO_2. The material once used for colorimetric reading was again employed for both colorimetric and gas chain method, since the pH changes usually before gas chain determination is made, thus giving a slightly higher alkalinity than its initial value.   During the second determination two colorimetric readings were taken and they were listed in the table (Table 2).   That the samples change their pH during the gas chain experiment is known from the colour tones at the beginning and at the end of each determination.   This may be due to the loss of CO_2 diffusing into the H gas which was introduced into the electrode to saturate the platinum black.   However such changes, if any, are very small, and in such cases the pH values were observed before and after each determination and two readings were averaged.

The standardization of my gas chain system showed in most cases E M F 515 m v (18°) and sometimes 514 m v (18°) against MICHAELIS' standard acetate solution.   Therefore this error measured carefully before and after each determination and the short E M F values were added to the E M F of the samples.   To test the stability of E M F of oyster blood 1.5 cc. of blood was taken from a specimen on June 6 th, 1928 and the E M F was estimated successively for 6 hours.

The results were as follows :

Case No.	E M F (19°)	Time elapsed
1	660.0	0 minute
2	659.8	2 ,,
3	658.7	5 ,,
4	660.3	10 ,,
5	658.8	20 ,,
6	660.0	30 ,,
7	660.0	1 hour
8	660.5	2 ,,
9	660.3	3 ,,
10	660.0	5 ,,
11	660.4	6 ,,
Mean	659.8	—

Having found by this determination the E M F of the oyster blood to be 659.8 m v in mean, 2 m v error of the gas chain was added to

this figure, thus obtaining the correct E M F 661.8 m v which corresponds to the pH 7.12 in this case.

1. Determination of the Blood pH.

Ten determinations have been made, the results being as follows.

TABLE 2.

No.	Change of E. M. F. (19°) (M. V) in the course of determination (time in minutes)								Error of gas chain (M. V)	E.M.F. of blood (M. V)	pH by EMF	pH by Indic.	Prot. error	Init. pH	No. of specimen
	0	1	3	5	10	20	30	Mean							
1	657.7	658.3	658.8	658.9	658.9	657.6	657.7	658.2	2.0	660.2	7.09	7.15	−0.06	7.10	36
2	671.5	671.8	671.5	671.5	671.4	671.4	671.4	671.5	2.0	673.5	7.33	7.30	+0.03	7.30	37
3	668.0	667.6	667.4	667.7	667.7	667.5	667.4	667.6	2.0	669.6	7.26	7.27	−0.01	7.10	38
4	672.0	672.0	672.0	672.0	672.0	672.0	672.0	672.0	2.0	674.0	7.33	7.32	+0.01	7.25	39
5	667.0	667.4	668.1	668.1	668.0	667.8	667.5	667.7	2.0	669.7	7.26	7.20	+0.06	7.10	40
6	666.1	666.4	666.0	666.2	666.9	666.4	666.0	666.2	2.0	668.2	7.22	7.25	−0.03	7.20	31
7	662.3	662.6	662.0	662.0	661.9	661.8	661.7	662.0	2.0	664.0	7.16	7.20	−0.04	7.00	32
8	660.7	660.1	659.8	660.0	660.0	660.0	660.0	660.1	2.0	662.1	7.12	7.10	+0.02	6.90	44
9	663.7	664.0	664.2	664.2	664.2	663.8	663.5	663.9	2.0	665.9	7.19	7.20	−0.01	7.10	48
10	671.9	672.1	672.0	671.8	671.8	671.8	671.8	671.9	2.0	673.9	7.33	7.33	0.00	7.20	51

By the 10 determinations cited above the E M F of the oyster blood is known to be favourably determined by the hydrogen electrode. Comparing the pH obtained by the indicator method with that of the electrometric method, the former values are larger than the latter in some cases, and vice versa in other cases. Therefore the protein error, i. e., the figures showing the difference of pH of the two methods, appears on both sides of zero with equal magnitude and equal frequency. The mean of this value has doubtful significance, being as small as pH −0.003. Consequently it can be said that the oyster blood hardly shows any protein error.

2. Determination of the pH of Pericardial Fluid.

The pH of the pericardial fluid was estimated 6 times on the following specimens, No. 23-29, which were employed in the determination by indicator.

TABLE 3.

No.	Change of E. M. F (19°) (M. V) in the course of determination (time in minutes)								Error of gas chain (M. V.)	E. M. F. of blood (M. V.)	pH		Prot. error	Init. pH	No. of specimen
	0	1	3	5	10	20	30	Mean			by E M F	by Indic			
1	663.5	664.1	663.6	663.3	663.3	—	664.0	663.6	3.0	666.6	7.21	7.16	+0.05	7.10	23
2	666.1	666.4	666.8	666.5	666.4	666.3	666.3	666.4	3.0	669.4	7.24	7.23	+0.01	7.10	25
3	661.1	661.4	661.5	661.8	661.8	661.6	661.5	661.6	3.0	664.6	7.17	7.14	−0.03	7.10	26
4	667.5	667.7	668.0	668.1	668.1	667.8	667.9	667.9	3.0	670.9	7.28	7.30	−0.02	7.30	27
5	658.8	658.3	658.2	658.2	658.2	658.1	658.1	658.3	2.5	660.8	7.10	7.11	−0.01	7.00	28
6	655.8	656.0	656.1	656.3	666.3	656.1	656.0	656.3	2.5	658.8	7.07	7.07	0.00	7.05	29

In the six determinations tabulated above the pH differences which exists between the gas chain method and the colorimetric method shows a close resemblance to that of the blood. Protein error appears in plus side in some cases or minus side in other cases, and in still other cases its value is near zero. The mean value indicates such an inappreciable figure as pH +0.002.

3. Determination of the pH of Body Fluid.

The pH of the body fluid was determined 5 times on the individuals No. 48-52 which were used in the indicator method.

As has been shown in the above table the protein error of body fluid is by far larger than that of the blood or pericardial fluid, amounting to pH −0.10 in mean.

TABLE 4.

No.	Change of E.M.F. (19°) (M.V.) in the course of determination (time in minutes)								Error of gas chain (M.V.)	E.M.F. of blood (M.V.)	pH by E.M.F	pH by Indic.	Prot. error	Init. pH	No. of specimen
	0	1	3	5	10	20	30	Mean							
1	652.6	652.0	652.3	652.5	651.9	651.8	651.8	652.1	2	0 654.1	6.98	7.2(	−0.22	7.20	48
2	667.5	667.7	668.0	668.0	667.5	667.5	667.4	667.6	2.0	669.6	7.26	7.30	−0.14	7.40	49
3	650.5	650.5	650.2	650.0	649.7	649.6	649.6	649.4	2.0	651.4	6.94	7.00	−0.05	6.90	50
4	664.4	664.4	664.4	664.4	663.8	663.5	663.5	664.1	2.0	666.1	7.19	7.20	−0.01	7.10	51
5	666.0	666.2	666.5	666.1	666.0	666.0	665.8	666.1	2.0	668.1	7.22	7.30	−0.08	7.30	52

4. Comparison of the pH of Blood, Pericardial and Body Fluid.

From what has just been mentioned it will be noticed that the pH values of the blood, pericardial fluid, and body fluid show a very close resemblance to one another, and that the protein error of the fluids are practically negligible, excepting the body fluid which has a marked protein error (pH −0.1). A comparison of the pH value of these fluids which were taken from one and the same individual, shows that the blood had a little higher pH than the pericardial fluid, which later surpassed the body fluid in the mean time.

In short the pH of these fluids closely resemble one another though the blood pH shows a little higher value than the other two, while there are no appreciable differences between the pericardial fluid and the body fluid. The pH of all these fluids shows a far lower value than the pH of sea water, indicating the existence of some slight gap between the oyster fluids and sea water.

C. THE CO₂-CONTENT OF THE BLOOD, AND PERICARDIAL FLUID.

Simultaneous determinations concerning the CO₂-content and the pH of blood and pericardial fluid were carried out 34 times on different normal individuals. The results were listed in the Table 5

TABLE 5.

No.	Air temp.	Water temp.	Specif. grav. of water	Water pH	pH		CO₂-content (vol. %)		Dimension of specimen			Date of experiment (1928)		
					Blood	Peric. fluid	Blood	Peric. fluid	Length (cm)	Breadth (cm)	Weight (gms)	Month	Date	Hour
1	27.0	23.0	23.00	8.10	7.25	7.05	4.11	3.39	15.0	11.0	600	VII	31	2.00 p m
2	,,	,,	,,	,,	6.90	6.80	3.50	3.49	10.5	8.5	346	,,	,,	3.00 ,,
3	,,	,,	,,	,,	7.50	7.10	4.19	4.09	13.5	8.0	495	,,	,,	3.40 ,,
4	,,	22.0	,,	,,	7.40	7.05	4.70	4.34	12.0	8.0	370	,,	,,	4.30 ,,
5	25.0	19.0	23.50	8.20	7.05	6.95	3.45	3.39	13.0	8.0	400	VIII	2	3.30 ,,
6	,,	,,	,,	,,	7.40	7.22	4.38	4.12	12.0	9.0	350	,,	,,	4.00 ,,
7	,,	,,	,,	,,	6.80	6.75	3.06	3.06	12.0	8.0	350	,,	,,	4.30 ,,
8	24.2	21.0	23.20	,,	7.65	7.60	4.87	4.35	13.0	10.5	480	VIII	4	8.00 a m
9	,,	,,	,,	,,	7.65	7.60	4.87	4.87	12.0	10.0	430	,,	,,	8.30 ,,
10	25.5	,,	,,	,,	7.58	7.52	4.40	4.40	12.0	6.5	530	,,	,,	9.00 ,,
11	25.6	,,	,,	,,	7.60	7.30	4.97	4.14	13.2	8.5	576	,,	,,	10.00 ,,
12	26.0	,,	,,	,,	7.60	7.35	4.39	4.36	12.2	8.5	330	,,	,,	11.00 ,,
13	26.5	,,	,,	,,	7.25	7.10	4.04	3.98	12.2	7.8	300	,,	,,	1.00 p m
14	,,	,,	,,	,,	7.00	6.90	3.47	3.34	12.2	9.5	350	,,	,,	1.30 ,,
15	,,	,,	,,	,,	6.80	6.80	3.29	3.29	11.0	8.5	460	,,	,,	2.30 ,,
16	26.10	20.3	23.60	,,	7.50	7.40	4.36	4.19	10.0	9.0	320	VIII	14	3.00 ,,
17	26.0	20.0	,,	,,	7.30	7.20	3.68	3.39	14.0	8.0	360	,,	,,	4.00 ,,
18	,,	20.5	,,	,,	7.42	7.40	4.59	4.33	11.0	8.0	505	,,	,,	8.30 a m
19	,,	,,	,,	,,	7.40	7.35	3.48	3.84	10.0	8.0	340	,,	,,	9.20 ,,
20	,,	,,	,,	,,	7.60	7.45	4.23	4.36	14.0	8.0	420	,,	,,	10.20 ,,
21	,,	24.5	22.80	8.05	6.90	6.75	3.72	3.24	16.0	9.0	475	IX	2	5.30 p m
22	28.0	24.3	,,	,,	7.10	7.00	3.90	3.70	16.0	10.0	450	,,	3	3.10 ,,
23	29.0	24.8	,,	,,	7.25	7.20	3.79	3.35	18.0	9.0	420	,,	4	1.30 ,,
24	25.0	24.2	22.90	,,	7.30	7.30	4.66	4.40	15.0	7.0	450	,,	15	2.00 ,,
25	,,	,,	,,	,,	7.10	7.05	4.20	3.58	16.0	8.0	400	,,	15	1.30 ,,
26	27.0	24.5	,,	,,	7.45	7.40	4.35	4.22	12.0	9.0	320	,,	10	9.00 a m
27	14.3	12.6	24.50	8.22	7.70	7.50	4.81	4.13	16.0	12.0	665	XI	19	8.30 ,,
28	15.0	12.6	,,	8.22	7.80	7.70	4.33	4.22	18.0	10.0	545	,,	,,	9.00 ,,
29	,,	12.8	,,	,,	7.50	7.50	4.90	4.25	17.0	8.0	600	,,	,,	9.30 ,,
30	,,	,,	,,	,,	7.70	7.50	5.11	4.76	16.0	8.0	480	,,	,,	10.00 ,,
31	15.2	,,	,,	,,	7.80	7.75	4.81	4.73	16.0	10.0	620	,,	,,	10.30 ,,
32	,,	,,	,,	,,	7.80	7.80	4.81	4.08	13.0	10.0	360	,,	,,	11.00 ,,
33	,,	,,	,,	,,	7.80	7.80	4.08	4.08	16.0	10.0	495	,,	,,	11.30 ,,
34	,,	12.9	,,	,,	7.30	7.20	4.62	4.62	20.0	11.0	630	,,	,,	12.00 n
Mean	—	—	—	—	7.39	7.28	4.22	4.00	13.8	8.6	448		,,	—

with the other factors observed at the time of experiment.

1. The CO₂-content of the Blood of Oyster.

The data given in the above table indicate that the CO₂-content of the blood of oyster ranges from a minimum value of 3.06 vol% to a maximum value of 5.11 vol%. The mean value has been found to be 4.22 vol%. The mean error of each determination showed a figure

of ± 0.50 and the mean error of the mean ± 0.008. And hence, so far as the present study is concerned, the CO_2-content is best represented by 4.22 ± 0.008 vol% (Table 5).

2.   The CO_2-content of the Pericardial Fluid of Oyster.

Thirty three determinations which have been made simultaneously on the same individuals as used in the blood experiment are given in Table 5. These results show that the CO_2-content of the pericardial fluid of the oyster is 4.87 vol% in the highest case, and the lowest content 3.06 vol%, showing the mean value to be 4.00 vol%. The mean error of each determination has been found to be ± 0.49 vol%, and the mean error of the mean to be ± 0.007 vol%. Consequently the best representative value of the CO_2-content of pericardial fluid becomes 4.00 ± 0.007 vol% (Table 5).

3.   The Comparison of the CO_2-content of the Blood and the Pericardial Fluid.

The mean value of the CO_2-content of the blood is larger than that of the pericardial fluid by some 0.22 vol%. This difference, though small, has been found in every determination and the CO_2-content of blood always exceeds that of the pericardial CO_2-content excepting a case of No. 20, where a converse relation is shown. As has been already stated, the pH of these two fluids also showed a little but constant difference in all cases, indicating a slight excess of the blood to the pericardial fluid. Therefore it is safe to conclude that there exists some definite quantitative relation between the pH and CO_2-content of these fluids and indeed we find that the alkalinity of 0.11 in pH corresponds to the CO_2-content of 0.22 vol% in the present case.

D.   THE RELATION WHICH EXISTS BETWEEN THE pH AND THE CO₂-CONTENT.

The CO_2-content in both the blood and pericardial fluid of the oyster, which value is closer to the surrounding sea water, indicates that the buffer value ought to be poor. Therefore the change of pH

produces a similar change in the CO_2-content, that is the increase in the CO_2-content brings about a rise of the pH. The relation of pH to CO_2-content, however, is interrelated to the third factor, the CO_2 pressure, as is well seen by the HENDERSON-HASSELBALCH's equation. So pH may be high when the CO_2 pressure is kept low, provided the CO_2-content remained unaltered, and conversely the pH may be low when the CO_2 pressure is kept high, even in case of the same CO_2-content.
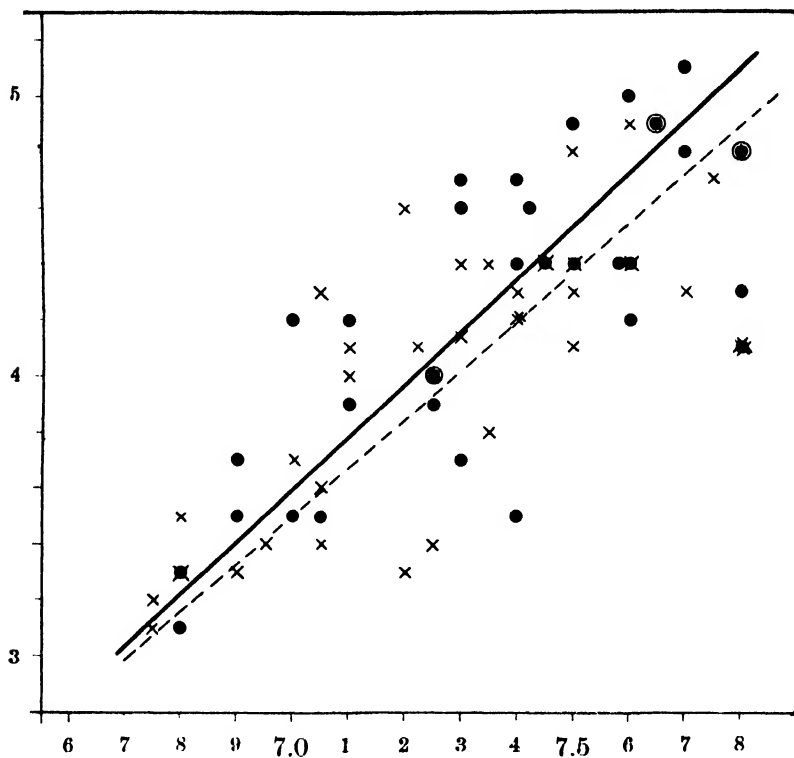

Fig. 2.   Ordinate—CO_2 vol%, Abscissa-pH.
Heavy line—Blood, Dotted line—pericardial fluid.
Dotts represent the blood, Crosses represent the pericardial fluid.

From the data (Table 5) obtained from the present experiment, the relation which exists between the pH and the CO_2-content can be expressed by the following two equations:

CO$_2$-content = 1.87   pH − 9.49 . . . . Blood.
CO$_2$-content = 1.31   pH − 6.49 . . . . Pericardial fluid.

These relations are illustrated in Fig. 2 in which the blood is represented by the continuous line and the pericardial fluid by the broken line. When the CO$_2$ pressure is disregarded or kept constant, therefore, the pH or CO$_2$-content of these fluids can be calculated from either of these two values. In reality, however, these fluids show a little buffer action and consequently these values are interrelated to the third factor, the CO$_2$ pressure, or in other words the relations cannot be represented by these simple equations given above. For instance from Fig. 2 we find that the CO$_2$-content of the blood having the pH 7.40 ranges between 3.50 vol% and 4.70 vol%, and similarly the pH of the blood having the CO$_2$-content 4.38 vol% ranges between the pH 7.40 and the pH 7.60. Such wide range of variations may be due to the buffer action of these fluids and the changes of the CO$_2$ pressure which corresponds to the denominator of the equation of HENDERSON-HASSELBALCH and enables the blood to show varying CO$_2$-content for a definite pH, or to show varying pH for a definite CO$_2$-content.

$$pH = pK' + \log \frac{(BHCO_3)}{(H_2CO_3)} \qquad (1)$$

According to AUSTIN and CULLEN[9] HENDERSON-HASSELBALCH's equation cited above (1) may be written as follows:

$$pH = pK' + \log \frac{(CO_2) - 0.1316 a pCO_2}{0.1316 a pCO_2} \qquad (2)$$

From equation (2) it follows that if the CO$_2$ pressure (pCO$_2$), absorption coefficient (a), and pK' are determined empirically, the question of the acid base balance of the blood and pericardial fluid can be solved from the above equation. However the complete solution of such a problem is reserved for future investigation, and in the present work I have shown merely the relation which exists between the pH and the CO$_2$-content, disregarding the CO$_2$ pressure. In Fig. 3 the normal variation of the pH and the CO$_2$-content of blood and pericardial fluid are shown by the graphic method of VAN SLYKE, giving the normal area of variation in both the blood and the pericardial fluid.
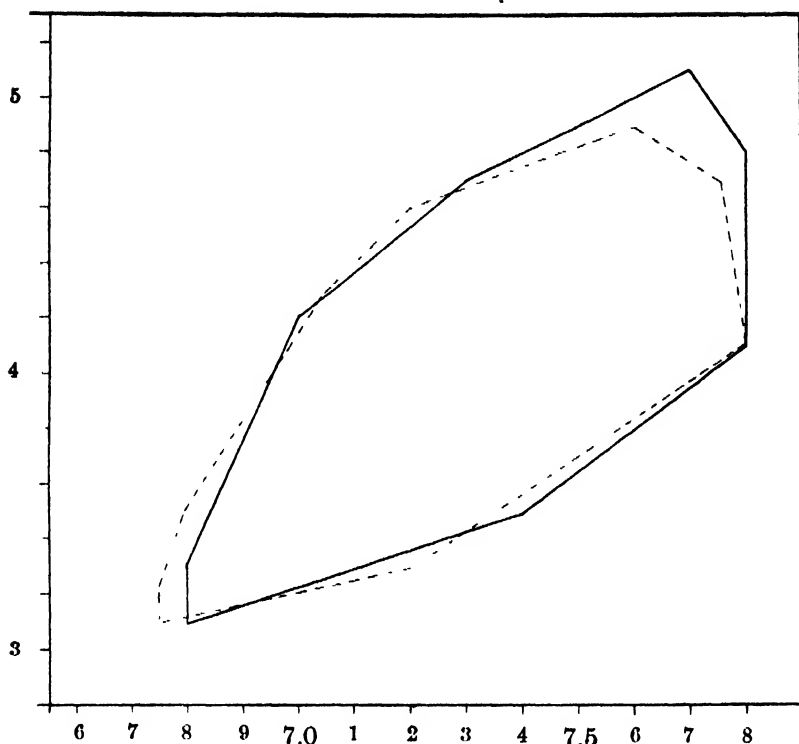
Fig. 3.   Ordinate-CO$_2$ vol%, Abscissa-pH.
          Continuos line represents the normal area of the blood.
          Dotted line represents the normal area of the pericardial fluid.

E. EXPERIMENTAL ALTERATION OF THE pH AND THE CO$_2$-CONTENT OF BLOOD AND PERICARDIAL FLUID 'IN VIVO'.

1. Acid Experiment.

With a view to determine the velocity of the effect of acidified breathing water upon the pH and the CO$_2$-content of blood and pericardial fluid five series of experiments were made on 28 different individuals, and the results are shown in Table 6 and 7.   The first two series were like experiments, using the sea water giving pH 5.00, the third experiment giving pH 3.00, and the fourth experiment

TABLE 6.

No. of exp.	No. of indiv.	Duration of exp.	Air temp. initial	Air temp. final	Water temp. initial	Water temp. final	Specif. grav. of sea water	pH of sea water	pH of breath. water initial	pH of breath. water final	pH Blood	pH Peric. fluid	CO₂ content Blood	CO₂ content Peric. fluid	Length	Breadth	Weight	Date	Hour
Exp. I	1	0 (m)	26.0	—	24.5	—	23.25	8.05	—	—	6.90	6.75	3.72	3.24	16.0	9.0	475	2	5.30 p m—
	2	15	26.0	26.0	24.8	25.0	22.80	"	5.00	5.30	6.30	6.25	6.70	6.32	14.0	7.0	450	9	10.35 a m—10.50 p m
	3	1 (hr)	26.5	27.0	24.5	24.7	23.25	"	"	5.50	6.20	6.20	7.58	6.37	14.5	8.0	580	2	11.00 a m—12.00 n
	4	2	28.0	27.0	23.7	25.3	23.27	"	"	5.60	6.30	6.40	6.26	5.79	15.0	8.0	450	2	2.10 a m— 4.10 p m
	5	6	27.6	28.0	23.6	26.8	23.28	"	"	5.40	6.40	6.20	3.50	4.19	14.0	8.0	440	2	8.00 a m— 2.10 p m
	6	12	26.5	26.5	23.3	26.5	—	"	"	5.60	6.60	6.50	3.51	3.47	16.0	8.0	516		5.00 a m— 5.00 p m
	7	24	28.0	26.0	24.5	26.7	23.25	"	"	5.60	6.60	6.65	10.64	10.49	13.0	9.0	438	1-2	4.30 p m— 4.30 p m
Exp. II	1	0 (m)	28.0	—	24.3	—	23.20	8.05	—	—	7.10	7.00	5.63	5.30	16.0	10.0	450	3	3.10 p m—
	2	30	28.2	28.2	24.3	25.0	23.22	"	"	5.20	6.10	6.00	5.48	4.81	13.5	8.5	480	3	2.45 p m— 3.15 p m
	3	1 (hr)	28.2	28.2	24.0	25.5	—	"	"	5.40	6.10	6.05	3.93	4.47	16.0	11.0	595	3	3.40 p m— 4.40 "
	4	2	26.2	27.2	24.0	25.7	23.20	"	"	5.50	6.55	6.50	4.88	4.28	17.0	10.0	525	3	11.10 a m— 1.10 a m
	5	6	25.0	28.0	25.8	23.8	23.25	"	"	5.70	6.75	6.40	4.64	4.24	14.5	11.0	430	3-4	8.20 " — 8.20 "
	6	12	25.8	25.5	23.8	25.2	—	"	"	5.70	6.50	6.70	4.64	2.84	16.0	8.5	460	3-4	9.30 " — 9.30 "
	7	24	25.0	25.0	25.3	25.2	23.30	"	"	5.60	6.50	6.40	4.41	5.62	14.5	10.0	596	4-6	9.00 " — 9.00 "
	8	48	25.7	25.0	25.3	25.0	—	"	"	5.40	6.45	6.40	9.09	9.38	15.0	10.0	590	4-6	8.40 " — 8.40 "
	9	48	25.2	26.0	24.9	25.0	23.30	"	"	5.50	6.45	6.40	9.32	7.15	14.0	9.0	515	4-6	5.00 p m— 5.00 p m
Exp. III	1	0 (m)	29.0	—	24.3	—	23.22	8.10	3.00	—	7.25	7.20	3.79	3.33	18.0	9.0	420	4	1.30 p m—
	2	1 (hr)	28.0	28.0	24.5	25.7	23.20	"	"	4.40	6.20	6.10	5.25	5.20	10.0	9.5	620	4	3.40 p m— 4.40 p m
	3	6	26.2	28.3	24.0	26.0	23.20	"	"	4.40	6.60	6.50	7.89	6.22	10.0	12.0	575	4	11.00 a m— 5.00 p m
Exp. IV	1	1 (hr)	27.0	—	24.5	—	22.90	8.10	1.20	1.60	6.40	6.42	5.63	5.12	16.0	10.0	430	10	9.10 a m—10.10 a m
	2	6	27.0	25.7	24.5	24.3	23.00	"	"	1.80	5.85	5.80	29.79	25.56	17.0	10.0	470		9.10 a m— 3.10 p m
	3	16	25.7	25.0	24.5	24.3	"	8.05	"	1.90	5.40	5.40	55.69	55.69	18.0	8.0	505		4.00 p m— 8.00 a m
	4	24	25.5	25.0	24.0	25.0	"	"	"	2.40	5.20	5.20	36.80	34.82	13.0	7.0	333	10-11	9.30 " — 9.30 "
	5	24	25.5	26.0	24.0	24.5	"	8.05	"	5.00	5.40	5.20	36.30	33.88	15.0	9.0	400	11-12	10.30 a m—10.30 a m
	6	24	26.0	27.0	24.0	25.8	"	"	"	3.70	5.20	5.20	55.08	45.34	17.0	14.0	450	11-12	2.30 p m— 2.30 p m

giving pH 1.20. The object of the fifth experiment was first to determine the susceptibility of the oyster to the low pH of sea water, and secondly to determine the lowest limit of blood pH at which the oyster can survive.

Experiments I and II.

a. *Conditions of the experiment.* The oyster was submitted to respiration in sea water the acidity of which was made pH 5.00 during 15 minutes to 48 hours. Experiment II was made in order to verify the results obtained from experiment I. For the purpose of comparison, an individual which had been living in the normal sea water was examined at the outset of experiment. The specific gravity of water used showed no marked change during the experiment, though it altered when HCl solution was added in order to decrease its pH. For instance a specific gravity of 1.0251 (10°) decreased roughly to 1.0239 (10°) when 100 cc. of n HCl solution was added for the purpose of changing its pH down to the neighbourhood of pH 1.20. The temperature of breathing water which was comparatively low at the beginning showed a slight rise at the end of each experiment, as the experiment was carried on under varying temperatures of the laboratory room.

b. *Change of the blood pH.* As will be noted in Table 6 that the high hydrogen ion concentration of the breathing water distinctly lowers the value of pH of the blood and the pericardial fluid. The normal reaction which has been found to be pH 7.24 in blood and 7.16 in pericardial fluid by the preceeding experiment, decreased as low as pH 6.40 and pH 6.36 (taking the mean of 14 determinations) respectively. Among the 14 determinations made in experiment I and II (excepting the normal individuals) the lowest value, pH 6.10 of blood was given by the specimens examined at 30 minutes and at 1 hour after the start of experiment II. The pH 6.75 was given by an individual examined at 12 hours after the start of experiment II, and pH 6.60 in two individuals examined at 12 and 24 hours after the beginning of experiment respectively.

At the outset of the experiment, I first anticipated that the degree of change of the pH of blood and pericardial fluid would be propor-

tional to the duration of their stay in the altered sea water, if the high hydrogen ion concentration of breathing water at all influences the reactions of these fluids. In reality a quite reversed relation was found. If, for instance, the pH of blood decreases from normal level to pH 6.30 in the course of 15 minutes, further decrease may be expected, if my anticipation were realized, by a lengthend exposure of the animal to the same condition. But instead an increase of pH was found, as was shown by the oysters which stayed for 24 hours in both experiments. This interesting relation will be discussed later.

What was found in the blood pH, was also true with the pericardial fluid. The lowest value of all of the pH of pericardial fluid was pH 6.00 which was given by the specimen (Exp. II) remaining for 1 hour. The relatively high value among 14 determinations has been found to be the pH 6.70 for 12 hours (Exp. II), pH 6.65, and pH 6.50 for 24 and 12 hours respectively. Thus the pH of pericardial fluid showed more profound change shortly after the oyster was placed in the altered sea water than those staying for a longer period.

At any rate it needs to be emphasized here that the pH of the blood and the pericardial fluid is changed within 15 minutes after the reaction of breathing water was altered (pH 5.00).

Of course there are several exceptions to the general statement given above, as one might anticipate in an experiment of this nature, and this irregularity may largely be due to the individual difference of the initial pH of these fluids.

    c. Change of the CO₂-content. Accompanying the change of the pH the CO_2-content of the blood and the pericardial fluid showed a marked change. In experiment I and II the mean value of the CO_2-content of the blood showed 5.99 vol% and 5.73 vol% respectively, and the pericardial fluid showed the mean value of 5.72 vol% and 5.34 vol% respectively, showing the increase of CO_2-content in these two experiments. Similar phenomena are also shown in Tables 4 and 5. These increases of CO_2-content in the course of experiment, however, are reciprocally proportional to the change of the pH in both experiments. In Experiment I, for instance, the CO_2-content decreased gradually for 6–12 hours, accompanied by a sudden increase at 27 hours. Such was also the case with Experiment II, as can be seen in the table. Why the CO_2-content once decreased during ex-

periment can be explained by the following fact : in the normal state of acid base equilibrium of the blood and the pericardial fluid, the alkali reserve, i. e. the bicarbonate, is well balanced with carbonic acid. If another acid, therefore, invades this buffer system, as in the case of the present investigation, the bicarbonate of the blood is correspondingly neutralized by that acid, evolving the carbonic acid and neutral salt. Since the carbonic acid evaporates from the surface of the gill and the neutral salt is excreted from the kidney, the potential alkali reserve of these fluids decreases temporarily to the minimum, thus showing a relatively low CO_2-content, as seen in the individuals No. 5 and No. 6 of experiment I and No. 3, No. 5, No. 6 of Experiment II. The increase of CO_2-content following this decrease which is noted for instance in No. 7 of Experiment I and No. 8 and No. 9 of Experiment II may therefore be attributed to the effect of high hydrogen ion concentration of breathing water.

 d. pH of breathing water. The sea water in which pH was made 5.00 at the outset of experiment, increased its value continuously during the course of experiment, till the end of the experiment. This increase of pH was observed 15 minutes after the introduction of the oyster to the sea water, as can be seen in case No. 2 of Experiment I, which showed an increase of pH 0.3 within 15 minutes. The degree of increase was proportional to the duration of the experiment, and such a high pH as 5.70 was attained at 12 to 24 or more hours later. The course of this increase may partly be due to the neutralization of acid by the alkali reserve of blood and pericardial fluid of the oyster, and partly to the dissolution of the oyster's shell, which in turn also neutralizes the high acidity of water.

Experiment III.

Still stronger acidity (pH 3.00) was tried on the three oysters to determine whether it causes a more marked change or not. The condition of experiment was the same as in the former experiment.

As is shown in Table 6, specimen No. 2 showed the blood reaction to be pH 6.20 and the pericardial fluid to be pH 6.10. In specimen No. 3 the blood showed pH 6.60 and the pericardial fluid pH 6.50. Though the alterations shown by these oysters are by no

means slight, nevertheless the acidity attained is no more pronounced for further increased acidity of breathing water, compared with the alterations shown by the Experiments I and II. The manner of the change of pH also agreed well with the two former experiments. An increase of pH with the elapse of time is also shown in the present experiment. The increase of CO_2-content however, occurs at 6th hour of the experiment contrasted with the occurrence at the 24th hour or later of the former experiments.

Increase of the pH of breathing water in this experiment was observed to be more marked than in the former experiments. An initial acidity of pH 3.00 increased to 4.40 at the end of the experiment, showing an increase of pH 1.40 in 1 to 6 hours.

Experiment IV.

Having found no further noticeable effect upon the blood and the pericardial fluid even when the acidity of sea water was considerably increased, the fourth experiment was tried, increasing the acidity of breathing water as high as pH 1.20. Six oysters were observed and the duration of experiment varied from 1 to 24 hours.

a. *Change of the pH.* The majority of oysters kept in this sea water of very strong acidity showed an exceedingly low value which had not been observed in any of the other experiments, and its value ranged from 5.20 to 6.40. The lowest value (pH 5.20) was obtained in the two specimens which were kept for 24 hours. The pH of the pericardial fluid also changed in a like manner to that of the blood, showing the pH 5.20 and the pH 6.42 respectively. It seems to worth special mention that the decrease in the value of pH in this experiment was strictly proportional to the duration of the experiment, contrasted with rather conflicting results obtained in the three former experiments. Since the pH of the pericardial fluid showed changes similar to that of the blood, no further comment is given.

b. *Change of the CO_2-content.* The change of the CO_2-content in the blood and the pericardial fluid in this experiment is a matter quite astonishing. Although the CO_2-content showed no marked change in individual No. 1, after 1 hour in the sea water, it increased later at a considerable rate. The CO_2-content of the blood ranged between

5.63 and 55.69 vol% with a mean value of 33.40 vol% in the course of experiment. The blood CO_2-content which was shown to be 5.63 vol% after 1 hour increased to 29.79 vol% at the end of the 6th hour, and reached the maximum of 55.69 vol% after 16 hours. After that, however, the CO_2-content increased no more, but instead it tended to decrease in the three individuals which were kept as long as 24 hours.

The pericardial fluid also behaved just in the same way as the blood. As has been already stated in the preceding page, the CO_2-content of the blood showed the pH 4.22 and the pericardial fluid pH 4.00 in the normal condition. Comparing these values to those obtained in this experiment one will be deeply impressed with an extraordinary increment of CO_2-content. Such prominent increase, however, was never confined to the above mentioned experiment, but it was also found in the other experiment which was carried out by me under a quite different condition, as will be stated later. As to the course of such marked increment, I shall discuss it in the following pages.

The marked decrease of acidity of the breathing water was noted in this experiment. The pH 1.20 at the beginning ascended from pH 1.60 to 5.00 during experimentation. The most prominent instance was shown by No. 5, in which the alkalinity rose up to pH 3.80 in the course of 24 hours. This increase of pH is assumed to be due to the neutralization of acid by the carbonate of the shell, as will be stated later.

  c. Condition of the oyster. In the natural habitat the oysters exhibit the opening and closing movement of shells quite regularly. When, however, they were brought to the aquarium, their normal behaviour appears to be much disturbed, and most of them close the shells tightly, exhibiting no movement for a long while. In my experiment therefore, I have chosen such individuals which exhibited their shell movement immediately after placing them in the jar. The oysters usually showed the shell movement at the beginning of the experiment, but sooner or later they opened the shells and weakened, and seemed as if narcotized, remaining opened. Especially those oysters which were tried with the sea water of stronger acidity became so weakened at the end of the experiment that only a strong agitation

would induce the oyster to close the shells.

Under the pH 5.00 the oyster showed no particularly unusual reaction unless kept in the water for 12 to 48 hours, after which the adductor muscle shows a relaxation. Under the pH 3.00 the experiment was carried out twice for a period not exceeding 6 hours, but no special changes were noted. However in the sea water which gives pH 1.20 a great many small bubbles, say 1 mm. in diameter, are evolved from the surface of the shell as soon as the oyster is transferred into it, and the water surface of the container became very foamy. The generation of gas, which is certainly due to the solution of the shell acted on by the high acidity of water, continues till the end of each experiment. The assumption that such acidity as instantly dissolves the shell will fatally affect the animal on the gill or mantle surface was fulfilled.

In such water as mentioned above the oyster discharges the sperms and contaminates the water. In such a case, the breathing water was renewed once or twice during the course of experiment. After 24 hours in this acid water the oyster becomes highly weakened. For instance in No. 4, the body colour became somewhat white and opaque, mantle showing no contraction by any stimulation, though the heart still continued faint pulsation. No. 6 behaved just like No. 4. In No. 5, which was the most active among the three, the body showed no colour change, mantle reacted well to the stimulation, and the heart exhibited a fine pulsation.

2.   Alkali Experiment.

To determine whether or not the pH of the blood, as well as of the pericardial fluid, can be increased as the result of strong alkalinity of the breathing water, and further, whether the CO₂-content increases when the blood pH is raised, was the object of the present experiment. Altogether three series of experiments involving 19 determinations have been undertaken. In the first, 9 oysters were placed into sea water having the pH 9.00. In the second series the breathing water was made equivalent to n/100 in NaOH concentration and 8 individuals were used, of which one oyster was examined in the normal state for the purpose obtaining a control. In the third series the alkalinity of

the water was made equivalent to n/50 in NaOH concentration.

The pH of the water, in these experiments became markedly low with the lapse of time. This may be of course due to the neutralization of alkali by the CO_2 evolved by the respiration of the oyster. Therefore it was almost impossible to keep a definite pH unaltered for long hours.

Experiment I.

As is well known, the alkalinity of natural sea water never exceeds pH 8.30. The sea water of the neighbourhood of our station generally varies from pH 8.00 to 8.20. It was therefore our expectation that the oyster, when exposed to the sea water having abnormally high alkalinity as was tried in the present experiment should show some change in the blood and pericardial fluid. The results of the present series of experiments are given in Table 7.

a. Change of the pH. In the control specimen (No. 1) the pH of the blood was 7.30, while the other specimens showed a great increase, giving a mean value of 8 determinations as pH 7.91. The lowest value was a pH 7.63 of No. 2, which was exposed for 15 minutes duration. The highest value of pH 8.40 was given by specimen No. 9, which remained for 24 hours. The pH of specimen No. 8, which stayed also for 24 hours, showed a value as high as pH 8.30. Although the oysters examined at the intervals of 30 minutes, 1, 2, 6, 12, hours, the increase of pH was not strictly proportional to the duration of exposure; nevertheless in general it may be stated that the pH is highest in the longest exposed specimen and lowest in the shortest exposed specimen. Therefore the effect of high pH water much resembles that of the low pH water at the point where the degree of change of blood pH is not proportional to the duration of exposure. However the effect of high and low pH differ in such a way that low pH often increases the blood pH in the course of time, while high pH shows no such peculiar relation.

The pH of the pericardial fluid was also increased as affected by the increased alkalinity of sea water, giving a mean pH of 7.75. The manner of change of pH is quite similar to that of the blood, and therefore no particular mention will be made of it.

b. Change of the CO₂-content. The CO_2-content of the blood showed in this case 4.98 vol% in mean. The lowest value of 1.45 vol% was given by individual No. 2, which was exposed for 15 minute duration. The highest value was 5.34 vol% in individual No. 7 which was examined at 24 hours. The other determinations, which were made at different intermediate periods showed some discrepancy but in general the CO_2-content tended to increase with the increase of time of exposure.

With regard to the pericardial fluid a similar relation was found, though some slight difference was shown in detail.

c. Change of the pH of breathing water. Although in most cases the same breathing water was used till the end of the experiment, in specimens No. 8 and No. 9 the water was renewed four times. The determination of pH was made just before the every renewal of water. At the end of one hour after the experiment commenced, namely just before the first renewal, the pH became approximately 8.20. However, one hour after the 1st renewal, that is, just before the second renewal, the pH again decreased to 8.80. Eighteen hours after the 2nd renewal, or just before the third renewal, the pH thrice decreased to 8.00. Twenty hours after the third renewal, i. e., just before the 4th renewal the pH became 8.35. Finally at the end of the experiment, that is, 24 hours after the beginning of experiment, the pH was found to show 9.00.

Experiment II.

In this experiment the pH was raised as high as the oyster could survive for any length of time. For this purpose n/100 solution of NaOH with sea water was employed. The pH of n/100 NaOH solution of distilled water is known to be pH 12.12 ordinarily, but the alkalinity somewhat decreases when the solution was made with sea water, unless some slight excess of NaOH is added. The colorimetric determination of the pH of this solution was almost impossible because of an inadequacy of a common indicator such as alizarine yellow or tropeolin 0 for sea water. Therefore the alkalinity was expressed here in terms of the normality of the solution instead of the pH.

a. Change of the pH. In this experiment too, the increase of

TABLE 7.

No. of indiv.	Duration of exp.	Air temp. initial	Air temp. final	Water temp. initial	Water temp. final	Specif. grav. of sea water	pH of sea water	pH of breath. Water initial	pH of breath. Water final	pH Blood	pH Peric. fluid	CO_2 content Blood	CO_2 content Peric. fluid	Length	Breadth	Weight	Date	Date (Sept. 1928) Hour
1	0 (m)	25.0	—	24.2	—	22.80	8.05	—	—	7.30	7.30	4.66	4.40	15.0	7.0	450	15	2.00 p m
2	15 ,,	26.0	26.0	24.8	25.0	,,	8.10	9.00	8.70	7.60	7.58	4.45	4.66	17.0	9.5	470	9	11.45 a m
3	30 ,,	27.0	27.0	25.0	25.3	,,	,,	,,	8.35	7.95	7.70	5.11	4.83	13.0	8.0	420	7	11.00 a m—11.30 a m
4	1 (hr)	26.5	27.0	25.0	25.5	,,	,,	,,	8.20	7.80	7.75	4.86	4.70	16.0	8.5	480	7	8.50 a m— 9.50 a m
5	2 ,,	26.5	27.0	25.0	25.0	,,	,,	,,	8.30	7.75	7.60	5.29	5.06	16.5	9.5	440	7	8.50 a m—10.50 a m
6	6 ,,	27.0	27.0	25.0	25.0	,,	,,	,,	8.05	7.70	7.70	5.00	5.00	13.0	8.0	360	7	11.10 a m— 5.10 p m
7	12 ,,	25.0	25.0	24.3	24.3	22.90	8.07	9.00	8.50	7.80	7.60	4.77	4.35	17.0	11.0	440	10-11	8.00 a m— 8.00 a m
8	24 ,,	29.0	26.3	24.8	25.5	,,	8.10	9.00	8.40	8.30	8.00	5.34	6.58	13.5	8.5	320	8-9	3.10 p m— 3.10 p m
9	24 ,,	29.0	26.3	24.8	25.2	,,	,,	,,	8.30	8.40	8.05	5.32	5.32	14.0	7.5	320	8-9	4.00 p m— 4.00 p m

TABLE 8.

No. of indiv.	Duration of exp.	Air temp. initial	Air temp. final	Water temp. initial	Water temp. final	Specif. grav. of sea water	pH of sea water	NaOH of breath. water initial	NaOH of breath. water final	pH Blood	pH Peric. fluid	CO_2 content Blood	CO_2 content Peric. fluid	Length	Breadth	Weight	Date	Date (Sept. 1928) Hour
1	0 (m)	25.0	—	24.2	—	22.80	8.05	n/100	—	7.10	7.05	4.20	3.58	16.0	8.0	400	15	1.50 p m
2	15	26.0	27.0	24.3	24.7	,,	,,		—	7.5	7.50	4.33	4.25	13.0	8.3	450	15	0.10 ,,
3	30	25.5	25.8	24.5	24.8	,,	,,		,,	7.80	7.70	2.79	3.35	15.0	7.5	480	12	7.55 a m
4	1 (hr)	26.0	28.0	24.2	24.5	,,	,,		,,	8.00	7.80	3.82	3.35	13.0	10.0	400	12	9.45 ,,
5	2	25.0	26.0	24.6	24.6	,,	,,		,,	8.20	8.80	6.30	5.09	15.0	9.5	360	12	7.25 ,,
6	6	24.9	26.0	24.6	24.9	,,	,,		,,	8.20	7.80	4.83	4.32	14.5	9.0	400	12	7.00 ,,
7	12	25.0	24.3	24.0	23.5	,,	,,		pH9.80	7.90	7.90	4.85	4.80	14.0	8.5	430	14-15	8.00 a m—8.00 p m
8	24	26.0	26.0	24.3	25.8	,,	,,		pH9.50	7.50	7.20	3.61	2.81	16.0	9.0	500	11-12	3.19 ,, —3.19 ,,

the blood pH due to high pH of water was clearly observed. The mean pH value of 7 determinations showed the pH 7.84, indicating a much higher value than that of the normal case. Because of the increased high alkalinity of breathing water the rise of the pH was naturally to be expected. For instance, in No. 4, No. 5 and No. 6, the pH rose as high as pH 8.00 or more, though in individuals No. 7 and No. 8 the results were somewhat reversed, showing rather low pH in spite of its long duration. Such contradiction as seen here may be attributed to the peculiar effect of the high pH of water, as will be shown by the susceptibility experiment. The pH of the pericardial fluid also showed a quite similar change as in the case of blood.

b. Change of the CO₂-content. The CO_2-content of the blood found in this experiment was less than that found in the former experiment, as will be seen by comparing its mean value (4.63 vol%) with that of the former case (4.98 vol%). Though it shows a slight increase from that of the control specimen (4.20 vol%), also from the mean value of this season (4.10 vol%), the maximum content which was found in individual No. 5 showed no less value than the normal one, indicating 6.30 vol%, yet the all other determinations showed a by far lower value than that of the former experiment, and the minimum content which was observed in the individual No. 3 reached such a low value as 2.79 vol%. Besides such general decrease, greater fluctuation of each determination characterised this experiment. A tendency of increase of pH as the duration of experiment lengthens was not found here.

In individuals No. 7 and No. 8 a peculiar change occurred, showing that a longer exposure to the experimental condition produced lesser CO_2-content than that of the shorter exposure. In general the pericardial fluid behaved almost similarly to the blood with minor exceptions which hardly need special comment.

c. pH of the breathing water. In specimen No. 6 the pH of water decreased to 8.80 at the fourth hour of the experiment, while in specimen No. 8 the pH of the water decreased as low as 7.70 at the 16th hour, twice at the 21st and 22nd hours, but thereafter it showed no change even when the water was altered three times.

Experiment III.

By the preceding two experiments it was made clear that the high pH of the breathing water materially affects the pH of the blood as well as the pericardial fluid within one hour. And the high pH of water used in experiment II seemed to have a more severe effect than that of Experiment I. Therefore the third experiment was undertaken with still higher pH to see whether it affects more severely the pH of these body fluids. In this experiment a n/50 NaOH solution was used as a breathing water. The two oysters were used, and the duration of experiment extended only to one hour. The results were as follows.

From an inspection of Table 9, p. 240, it will be noticed that the pH of the blood and the pericardial fluid of individual No. 1 was raised higher than it was ever seen in the other experiment, while No. 2, on the contrary, remained quite unaltered or only very slightly so. Such conflicting results produced by the oysters which were kept under the same condition was observed already in Experiment II (individual No. 8), and further examples will be given in the experiment of susceptibility together with some interpretations regarding this curious phenomena.

The mean value of the CO_2-content has been found to show an intermediate magnitude (4.53 vol%) of the former two experiments, showing but a slight increase when compared with the mean value of this season (4.10 vol%). Thus no distinct parallel change is manifested accompanying the alteration of pH. This peculiar behaviour of CO_2 just stated, relative to the change of pH, can be understood when the cause of the pH change in this instance is fully explained, which will appear later in the discussion.

Condition of the oyster in the alkali experiments. In the three alkali experiments above described, the oyster behaved more or less differently from the oysters subjected to the acid experiments. First, the weakness due to an altered unnatural condition was not so marked in alkali as in acid. Secondly, the oyster did not discharge the sperms at all, in contradiction to the case of acid experiment. Thirdly, the oyster excreted the faeces as in the natural water, while this has never been found in the acid water. The pulsation of the heart

apparently showed no change in spite of the long exposure to the high alkali water.

3. Susceptibility Experiment.

The oysters kept in the acid or alkali breathing water showed an instantaneous reaction of either decreasing or increasing the pH of the blood as well as of pericardial fluid. The next problem to be solved was the maximum rise of pH of internal medium ' in vivo ' together with the durability of the oyster against such a changed condition internally as well as externally.

For this purpose the acidity was made pH 1.20, and the alkalinity to n/100 solution of NaOH. The oysters were kept in these media for 48 hours. Three individuals for the acid experiment and five individuals for the alkali experiment were used. In this experiment the CO_2-content of breathing water was especially observed.

a. Susceptibility for the acid water.

After an immersion for 48 hours in the acid water as shown in Table 10, the pH of the blood was lowered conspicuously, showing in the three individuals designated A, B, and C, that the pH became 4.90, 4.80, and 5.40 respectively. From the fact that such low pH as attained here has hitherto never been observed, a severe effect of breathing water upon the oyster is evident. At the end of exposure for 48 hours in this high acid water the oysters were found to be in the following states :

Oyster A, shells widely opened, which were never closed even when strongly agitated ; mantle did not contract by stimulation, heart ceased its pulsation, thus showing a perfect death of the animal.

Oyster B, in the same state as oyster A.

Oyster C, although this individual showed extreme weakness during 48 hours it surely survived, since the margin of the mantle contracted by a stimulation with a needle, and the heart showed a feeble but perceptible pulsation.

The pH of the pericardial fluid showed a fine parallel change to that of the blood, except with the specimen A, which showed some-

TABLE 9.

No. of indiv.	Duration of exp.	Air temp. initial	Air temp. final	Water temp. initial	Water temp. final	Specific grav. of sea water	pH of sea water	NaOH of breath. water initial	NaOH of breath. water final	pH Blood	pH Peric. fluid	CO₂ content Blood	CO₂ content Peric. fluid	Dimension of specimen Length	Dimension of specimen Breadth	Dimension of specimen Weight	Date	Date (Sept. 1928) Hour
1	1 (hr)	23.5	24.3	2.40	24.0	22.80	8.10	$\frac{n}{50}$	—	8.45	8.45	4.71	4.33	14.0	7.5	440	16	9.45 a m–10.45 a m
2	1 "	23.8	24.7	2.40	24.0	"	8.10	"	—	7.60	7.50	4.35	3.81	14.0	8.0	420	16	11.00 a m–12.00 n

TABLE 10.

Exp.	Sign of fish	Air temp. initial	Air temp. final	Water temp. initial	Water temp. final	Specific grav. of sea water	pH of water original	pH of water initial	pH of water final	pH Blood	pH Peric. fluid	CO₂ content (vol %) Blood	CO₂ content (vol %) Peric. fluid	CO₂ content (vol %) water	Dimension of specimen Length	Dimension of specimen Breadth	Dimension of specimen Weight	Date	Date (Sept. 1928) Hour
For acid 48 hours	A	26.0	26.2	24.5	25.6	22.80	8.07	$\frac{n}{100}$	2.20	4.90	6.60	32.00	35.54	37.52	14.5	9.0	370	12–14	11.40 a m–11.40 a m
	B	26.0	26.2	24.5	25.5	"	8.07	$\frac{n}{100}$	4.40	4.80	4.60	30.51	27.76	16.52	12.0	7.0	390	"	0.10 p m–0.10 p m
	C	26.0	29.0	24.5	25.6	"	8.07	$\frac{n}{100}$	4.60	5.40	5.40	41.86	42.62	49.77	15.0	7.0	390	"	4.40 p m–4.40 p m
For alkali 48 hours	A	26.0	26.0	24.5	25.3	22.80	8.07	$\frac{n}{100}$	—	7.00	6.80	4.32	3.70	2.18	14.0	7.0	410	12–14	11.40 a m–11.40 a m
	B	26.0	26.0	24.5	25.3	"	8.07	$\frac{n}{100}$	—	8.40	8.40	—	5.52	2.30	13.0	7.0	380	"	"
	C	26.0	26.0	24.5	25.3	"	8.07	$\frac{n}{100}$	—	8.00	8.00	4.72	4.72	2.25	13.5	8.0	390	"	3.30 p m–3.30 p m
	D	25.0	22.0	24.7	21.6	23.20	8.10	$\frac{n}{100}$	—	7.65	7.60	3.16	3.16	1.23	14.2	9.0	340	15–17	9.00 a m–9.30 a m
	E	25.0	22.0	24.7	21.6	"	8.10	$\frac{n}{100}$	—	7.50	7.40	3.92	3.79	1.54	12.4	7.0	330	"	"

what high pH. As the pH of the blood and pericardial fluid underwent such a marked change as above mentioned, the whole body tissue of oyster might be assumed to have been bathed in a highly acidic fluid, at least at the end of the present experiment.

The change of CO_2-content of internal medium in this experiment verifies the results obtained from the former experiments. The CO_2-content of the blood of the oyster C, which still survived at the end of the experiment showed the highest content, with a value of 41.86 vol%. The remaining two individuals, i. e., A and B, showed it to be 32.00 and 30.51 vol% respectively, showing that the respiration of the animal in the acidified water raises its CO_2-content in a very distinct manner. The CO_2-content of pericardial fluid also showed a similar increase to that of the blood. The CO_2-content of breathing water showed a distinct decrease when HCl was added at the start of the experiment, giving as low a value as 1.99 vol%. But it increased at a rapid rate till the end of the experiment, and finally reached 37.52 vol%, 46.52 vol%, and 49.77 vol% in A, B, and C respectively. This increase was thus most marked in C and was least marked in A, and the rate of the increase was nearly 25 fold in the former and nearly 19 fold in the latter. The cause of this increase undoubtedly depends on the dissolved $CaCO_3$ of the oyster shell in the breathing water as is indicated by an evolvement of bubbles on the surface of the shell.

The increase of pH of the breathing sea water during the course of the experiment has been observed very accurately. It increased to pH 2.20 in the specimen A, pH 4.40 in B, and pH 4.60 in C. Such increase may in part be due to the neutralization of acidity by various body fluids of the oyster, but probably the greater part of it is due to the dissolved shell carbonate. During the experiment the breathing water was renewed at the 20th hour, when the acidity was pH 1.60 in A, pH 1.50 in B, and pH 2.00 in C. Again at the 30th hour it was once more renewed when the acidity was pH 1.90 in A, and pH 2.20 in both B and C.

One of the noteworthy facts in this experiment was that the oyster never excreted the faeces, contrarily to the alkali experiment in which the oyster excreted the faeces as in the natural water. Another noteworthy fact was that the heart remained swollen, filling the pericardial cavity, while in the alkali experiment it shrank into a small lump.

On the whole, the oyster survived as long as the blood pH was held above pH 5.40 and only a slight further decrease to pH 4.80 or 4.90 was fatal to the oyster. Therefore so far as the present experiment is concerned the oyster can survive if the pH of the blood does not fall below the pH 5.00.

b.   Susceptibility for the alkali water.

The behaviour of the oyster kept in the alkali water of concentration of n/50 to n/100 of NaOH varies considerably, for instance the oysters kept for 12 hours or more in the alkali water mentioned above show no change in some cases (No. 7 and No. 8 in Table 8, and No. 2 in Table 9). Among the five individuals employed in this experiment a marked rise of pH was noted with B and C, and no change with A, D, and E at the end of 24 hours. Therefore it may be said that the behaviour of the internal medium of the oyster differs highly according to the individuals. So that the contradictory results found in the present experiment as well as in the previous experiments can not be accidental, and I shall take up this matter in the discussion.

The oysters kept in this alkali water then survived well, and showed relatively a little weakness, in contrast with the oysters kept in the acid water. Among the five specimens, A and B showed a moderate weakness, and the heart shrank a little, continuing a slow pulsation. In specimen C the heart shrank to such an extent that the blood was with difficulty collected though the mantle distinctly responded to the stimulation. Specimen D and E showed no marked weakness, and furthermore showed very active pulsation of the heart.

The CO_2-content of the blood in this experiment showed 4.72 vol% in maximum, 3.16 vol% in minimum, and 4.03 vol% in mean. This mean is somewhat low when compared with the mean value obtained from the normal oysters of this season (4.10 vol%). In general a slight decrease of CO_2-content is noted with the oyster kept in the strong alkali water. The mean value of CO_2-content was slightly higher in the pericardial fluid than in the blood, though both showed a decrease under the alkali condition.

The CO_2-content of the breathing water was about 4.00 vol% at

the outset of the experiment, which however, decreased to as low a value as 1.54–2.30 vol% at the end of the experiment. Concerning the real cause of this decrease just given, we must await further future investigation.

The breathing water was changed four times (after 7, 20, 35, and 45 hours) during the experiment, yet the pH showed a marked decrease in each case as will be seen from the following:

Renewal of water	Specimen		
	A	B	C
Start	n/100	n/100	n/100
After ca. 7 hours	pH 8.50	pH 10.00	pH 10.00
After ca. 20 hours	pH 8.45	pH 8.35	pH 8.35
After ca. 35 hours	pH 8.75	pH 8.70	pH 8.70
After ca. 45 hours	pH 8.40	pH 8.40	pH 8.40

The reaction of water is thus subjected to a marked change. As to the cause of this change, though the metabolism of the oyster itself undoubtedly plays an important rôle, yet the effect of respiration and photosynthesis of many organisms which are attached to the shell of the oyster can not be neglected.

In short the oysters survived well under such alkalinity as tried in this experiment, and none of the individuals died till after 48 hours. Consequently it may be concluded that the high alkalinity around pH 12.00 (more correctly n/100 NaOH solution) does not affect the oyster so seriously as the pH 1.20 (neighbourhood of n/100), which was fatal without exception for the same time limit.

4. Air Exposing Experiment.

COLLIP's[4] interesting finding that the *Mya arenaria* increases the CO₂-content of the coelomic fluid when it was exposed in the air, induced the author to make a similar test with the hope to determine whether the CO₂-content of the blood and the pericardial fluid of the oyster increases by the same cause or not. For this purpose a series of experiments was conducted with the oyster. The oysters were exposed to the air, placing them on the floor of the laboratory. The

determinations were made 9 times on nine individuals, and one of them was examined immediately after it was taken out from its native water, in order to secure the normal data. The remaining 7 individuals were examined once for twenty-four hours during the successive 7 days. The pH of both blood and pericardial fluid was examined simultaneously.

TABLE 11.

Duration of exp. 0	Air temp.		pH		CO$_2$ content (vol %)		Dimension of specimen			Date (Sept. 1928)	
	initial	final	Blood	Peric. fluid	Blood	Peric. fluid	Length (cm)	Breadth (cm)	Weight (gm)	Date	Hour
1	27.0	—	7.45	7.40	4.35	4.22	12.0	9.0	320	10	9.00 a m
2	27.0	26.3	7.20	7.15	7.22	7.48	17.0	9.5	570	9	4.30 p m
3	27.5	27.0	7.00	6.90	11.46	11.28	15.0	8.9	380	4-6	11.00 a m–11.00 a m
4	27.5	29.0	7.05	7.00	11.28	11.23	16.0	8.5	430	4-7	,,
5	27.5	26.0	6.90	6.90	22.48	20.74	14.5	9.0	340	4-8	,,
6	27.5	26.1	6.80	6.70	33.50	32.41	14.0	10.0	420	4-9	,,
7	27.5	25.8	6.80	6.80	38.00	34.10	16.0	8.5	450	4-10	,,
8	26.1	22.0	6.60	6.70	40.19	34.56	13.5	8.5	370	12-19	3.00 p m– 3.00 p m
9	28.0	24.0	6.70	6.65	35.00	31.05	13.0	9.0	390	9-16	4.00 p m– 4.00 p m

a. Change of the pH.

i. *Change of the pH of blood.* Control specimen (No. 1) showed its blood alkalinity to be pH 7.45, indicating the highest value in this experiment. In specimen No. 2 which was exposed for 24 hours to the air, the blood pH has been found to be 7.20. Specimen No. 3, which was exposed for 48 hours, showed further decrease in pH of the blood, giving 7.00. The specimen No. 4, happened to show a little higher value of pH than the former case at the end of the 3rd day. The specimen No. 5, however, showed the ordinary decrease, giving pH 6.90 on the 4th day. The lowering of the pH continued till the 7th day, attaining to a minimum value pH 6.60. After the 7th day additional determinations were made twice, and one showed it to be pH 6.60 while the other showed pH 6.70. At any rate the pH of the blood showed a distinct decrease in the course of 7 days, and finally it reached such a low value as is never met with in normal condition.

ii. *Change of the pH of pericardial fluid.* The change of the pH of pericardial fluid behaved similarly to that of the blood pH in general. The degree of alkalinity of pericardial fluid (pH 6.91), however, showed a smaller value in terms of pH compared with the mean pH of the blood (pH 7.17).

In short the pH of these fluids stays well within the normal range until the 4th day, after which it became exceedingly low, beyond the normal range. The value of the pH at five days or later indicates more alkaline reaction than that given by the acid experiments I and II. Therefore it was my expectation to find a lesser amount of CO_2-content for the value of pH given after five days of experiment, because in the light of the acid expeirment any marked increase of CO_2-content cannot be expected for such a range of pH. Contrary to my expectation an astonishing increase of CO_2-content was found, thus showing the presence of a remarkable difference between the air exposing and the acid experiment, as will be stated in the following lines.

b. Change of the CO_2-content.

i. *Change of the CO_2-content of the blood.* Quite contrary to the change of the pH, the CO_2-content of the blood showed a great increase. At the outset of the experiment the control specimen (No. 1) showed the CO_2-content to be 4.35 vol%, a most representative value. It became, however, 7.22 vol% after being exposed for 1 day, indicating an increase of 1.66 fold. Then it increased to 11.46 vol% at the end of 2nd day, or 2.63 fold of the initial value. Three days after, however, it showed no increase, or rather a slight decrease. This decrease. however, indicates individual difference. At the end of the 4th day CO_2-content promptly increased up to an amount of 22.48 vol%, showing an increase of 5.17 fold. On the fifth day it sprang up to 33.50 vol% or 7.70 fold of the original value.

The increase continued further until it showed 38.00 vol% on the 6th day and 40.19 vol% on the 7th day, indicating an increase of 8.73 and 9.23 fold respectively. In another determination on the 7th day it was found to be 35.00 vol%. Therefore the maximum value attained at the end of a week is 40.19 vol%.
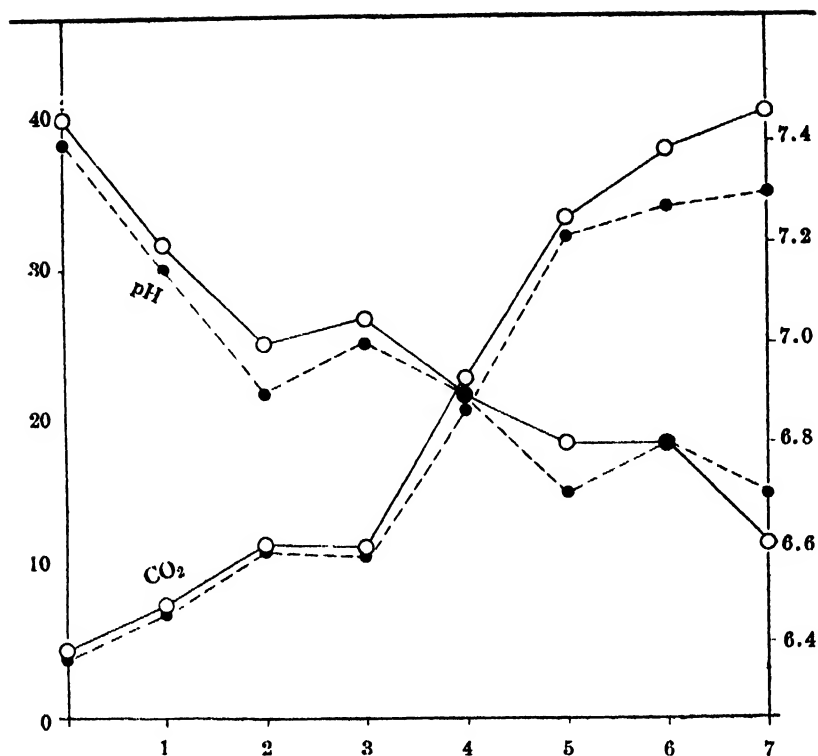
Fig. 4. Ordinate (Left side) — CO_2 vol. %.
         Ordinate (Right side) — pH.
         Abscissa — Date.
         The circles represent the blood.
         The dotts represent the pericardial fluid.

From an inspection of the accompaying figure it will be observed that the rate of increase in the course of a week is most rapid on the 4th and 5th days, and during the first three days and the last two days the increase is less rapid.

ii. *Change of the CO_2-content of the pericardial fluid.* The CO_2-content of the pericardial fluid showed always a lesser value throughout the entire course of experiment, and other marked differences were found.

From the present experiment it is clear that the pH and the CO_2-content of the internal medium of the oyster are changed definitely

when the animal is exposed to the atmospheric air for some days. A similar change was observed also when the oyster was forced to respire in the strong acidic water (Acid Exp. I and II). Therefore it can be said that the oyster manifests a like physiological response either when it was exposed to the air or to the acidic water. But the manner of change in both cases is not entirely the same.

In the oysters kept in the strong acid water pH of the internal medium decreases very rapidly. For instance it reached as low a pH as 6.10 to 6.30 within 30 minutes, when kept in the water of the pH 5.00. The pH tends to increase very slightly in course of time (Acid Exp. I. II), while the lowest pH attained by a survived oyster in the Acid Exp. IV was pH 5.20. On the other hand the decrease of pH due to the air exposing is very slow and the pH decreases regulary with the lapse of time. Furthermore the change is much milder than that shown by the acid experiment, reaching to a minimum pH 6.60, which is far higher than the minimum given by the acid experiment.

The change of the CO_2-content also shows distinct difference between the acid experiment and the air exposing experiment.

In the acid experiment the CO_2-content shows a marked increase which is accompanied by the increased acidity of the blood, but the strength of the acidity is not always proportional to the CO_2-content (Acid Exp. I and II). When however the pH fell below 6.00 the CO_2-content abruptly sprang up to an astonishing amount of 55.69 vol% both in the blood and in the pericardial fluid (Acid Exp. IV and V). Whereas in the course of air exposing experiment the CO_2-content increases reciprocally to the pH value, as can be clearly seen in Fig 4.

Furthermore the maximum CO_2-content caused by the air exposing attained but 40.19 vol% in blood, while the maximum CO_2-content caused by the strong acid water exceeds this value to a considerable degree as already mentioned.

According to the results of COLLIP[4] the CO_2-content of the coelomic fluid of *Mya arenaria* increases considerably with time, when it was exposed to atmospheric air. His data shows that the normal CO_2-content of the coelomic fluid of this animal ranges from 6.5 to 11.2 vol%. After the exposure for 24 hours it increased to 28.2 to

38.0 vol% indicating an increase of 3 to 4 [fold. This increase continued further, showing 65–69 vol% after two days, 78.2 vol% after
three days and at last it reached a maximum value of 105.0 vol%
after 4 days, showing the increase of nearly 10 fold.

Comparing these results with the present case the following differences will be noticed : (1) The normal CO_2-content of *Mya* is much
higher than that of the oyster. (2) The rate of increase of CO_2-content
is more rapid in *Mya* than in the oyster. (3) The highest CO_2-content
attained is 105 vol% in *Mya* at the end of 4th day while in the
oyster it was only 55.69 vol% at the end of 7th day.

DISCUSSION.

The fact that the heart of the oyster survives highly actively in
the ordinary sea water as long as 14 days, rendered the author to
entertain a question whether there may exist some close relation
between the pH of sea water on one hand and the pericardial fluid
and the blood on the other. As is generally known the alkalinity of
sea water is mostly found to be above the pH 8.00, showing a higher
value than the pH of the blood of most animals. But the pH of
the blood in some marine lower animals show a fairly high value,
as is reported in my previous paper[2]. For instance, in *Arca* a
large bivalve, the blood pH was shown to be pH 7.75 indicating close
approximation to that of sea water. Therefore one is tempted to
assume that the pH of the sea water is the most favourable medium
for the organs of most marine animals including the oyster heart. In
fact, however, the pH of the blood and pericardial fluid of oyster is
usually much lower than that of sea water when it is compared with that
of *Arca* (pH 7.75) or *Caudina* (pH 7.79) and rather approximates that
of some fishes (*Salmo iridius* pH 7.28, *leuciscus hakuensis* pH 7.35)
(2) or even mammals (neighbourhood of pH 7.40). The protein error
of the blood and pericardial fluid was found to be negligible owing
to the scantiness of protein or salts in these fluids, although there is
another possibility of the existence of two different kinds of proteins,
which gives the opposite error to an indicator, thus minimizing the
protein or salt errors (7).

COLLIP finds that the CO_2-content of the coelomic fluid of Pelecy-

poda mollusca, *Mya arenaria*, ranges from 6.5 to 10.2 vol% when it was equilibrated with atmospheric air. In many other pelecypoda species such as *Saxidomus*, *Macoma*, *Mytilus*, and *Cardium* the CO_2-content of coelomic fluid showed nearly a similar value, excepting *Paphia* in which this value is high as 29 vol%. The author[2] found that the CO_2-content of the blood of *Arca* (a bivalve) and *Caudina* (an echinodermata) to be 6.3 vol% and 8.5 vol% respectively. In the present investigation the CO_2-content of the blood and pericardial fluid was found to be 4.22 vol% and 4.00 vol% respectively. As will be seen from these figures the CO_2-content of the blood and the pericardial fluid of the oyster is very low as compared with the blood and the coelomic fluid of other marine lower forms given in the above. The CO_2-content of sea water which was often exammined during the course of the present experiment varied in the range of 3.95 to 4.20 vol% thus showing a close approximation to that of the blood and the pericardial fluid of the oyster. In spite of such resemblance of alkali reserve there exists a distinct difference between sea water and the fluids of the oyster with regard to its pH value. The reason why such difference arises may be explained by a difference of the tension of free CO_2 in these fluids. In the blood or the pericardial fluid the alkalinity i. e. combined carbondioxide will be oppressed by the presence of a greater amount of free CO_2 which was produced from the tissue, and the reaction will be more or less shifted to the acid side. While as sea water has no such CO_2 its pH will be kept higher than that of the blood or pericardial fluid.

    I now wish to give some comment concerning the behaviour of these fluids to the altered condition of sea water.

    As has been already mentioned, the oyster alters blood pH as well as the pH of the pericardial fluid distinctly, in responce to the pH change of breathing water. In the acid experiments it was found that the speed of this change is very rapid and it can be recognized as soon as within 30 or even 15 minutes after the animals are placed under an altered condition. For instance an individual (No. 2) decreased its blood pH from normal value to pH 6.10 within 30 minutes when it was immersed in the acid water (pH 5.00). This decrease may be regarded as a change due to the influence of the low pH of external medium. Consequently it may be allowable to

believe there occurs a penetration of H ion from breathing water into the blood as well as to the pericardial fluid. The penetration of acid into living tissue is not yet fully studied, but according to TAYLOR[1] the acid can penetrate through a living membrane either in the form of an undissociated molecule or by the simultaneous passage of H ion and anion. Therefore in my experiment also the HCl in water would have penetrated into the animal body either in form of ion or molecule.

It was noted in the Acid Experiments I, II, and IV that the pH change of internal medium (blood and pericardial fluid) gives conflicting results. That is, in Acid Experiments I and II the pH of internal medium tends to increase for 12 or 24 hours, while in Acid Experiment IV the pH of internal medium rapidly decreases with time. These contradictory results may have been produced by a difference of the pH in the breathing water in these two experiments. In the Experiment I and II the pH of the water was not so high as that of the Experiment IV. In Experiment I and II the pH decreased immediately by a rapid penetration of acid, which in turn from the neutralization of the acidity showed a temporary increase of the pH of internal medium later. While in Experiment IV, because of very strong acidity of breathing water, the alkali reserve of the internal medium could not defend a decrease of pH, thus producing a progressive decrease of pH in internal medium with the course of time.

The next subject to be discussed is a more important one, i. e. the change of the CO_2-content. The CO_2-content in the blood, that is alkali reserve, is known to have an intimate relation with the respiration, particulary upon the socalled phenomena of 'acidosis' in higer animals. In lower animals, however, this relation has been studied very little until COLLIP's result[4,5] was published. The relation of my own observations on the oyster to the study of COLLIP and further to the more general subject of the acid base problem will be discussed in the following.

Remarkable findings in my acid experiment are first that the CO_2-content of the internal medium decreases at first and then increases when the animal is immersed in the moderate acid water (Acid Exp. I, II), and secondly that the CO_2-content of the internal medium rises gradually and continuously to a great extent, when the animal is

subjected to respire in the water of strong acidity (Acid Exp. III, IV). Thus the question centers on the significance of the difference found between the moderate and intense acid experiments.

The decrease of CO_2-content in the beginning of the experiment of moderate strength of acid has been emphasized in each experiment, and it was attributed to the neutralization of proper alkali reserve due to HCl which has rapidly invaded the internal medium from the outer medium. The increase shown in the later stage may also be due to the same nature as the increase observed in the Exp. IV, where much stronger acid was used.

When the oysters were submitted to respire in the low pH water, the HCl diffused into the blood, probably through the gill membrane at first and finally from all over the surface of the mantle. If the blood becomes thus acidic, the tissue which is bathed in the blood and also in the pericardial fluid will become more and more acidic in the course of time. In the beginning of acid invation the alkali reserve of the blood or possibly the sodium bicarbonate will first neutralize it. But it is very evident, however, that the quantity of alkali contained in the blood is insufficient contrasted with the intense acidity of the breathing water. It is an extraordinary fact that in spite of such poorness of the alkali reserve the blood is able to counteract for the decrease of its pH to such an extent as to enable the oysters to survive as long as 24 to 48 hours under an acidity as high as pH 1.20. Such unique control of acid base balance can not be maintained unless there were a powerful regulative ability in the body of the oyster. The ability just stated is manifested by the important role played by the shell carbonate of the oyster.

The fact that the CO_2-content of the internal medium of the oyster, which shows a normal to be 4.00-4.22 vol% increased to 55.69 vol% already has been emphasized in the appropriate places. Such an immense increase can not be explained unless we presume an existence of a vast quantity of alkali reserve in the body of the oyster. This large quantity of the alkali reserve must be attributed to the shell of the oyster. This idea was first maintained by COLLIP[4] in his study of the anerobic respiration of *Mya arenaria*. In his case the increased carbon dioxide in the blood raises the blood acidity and in consequence of this acidity the calcium carbonate of shell dissolves in the blood,

and increases the CO_2 reserve in the blood. For explanation of my results the utilization of shell carbonate against increased acidity has been also assumed. As to the cause of acidity, however, a different interpretation is needed, since in place of the carbonic acid in COLLIP's case invasion of HCl from water is responsible. The following equation will illustrate these relations just stated.

$$CaCO_3 + 2\,HCl = CaCl_2 + CO_2 + H_2O$$
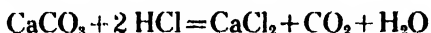
By this reaction the carbon dioxide and the calcium chloride will be increased in the blood. But as the carbon dioxide will be immediately dissolved and will act as an acid the shell carbonate will be furthermore dissolved. The subsequent formation of calcium bicarbonate from calcium carbonate will be seen from the following equation.

$$CaCO_3 + H_2CO_3 = Ca(HCO_3)_2 \ldots \text{(COLLIP's equation)}$$
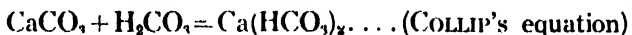
The calcium bicarbonate thus formed will serve as an excellent alkali reserve.

As will be seen from the above two equations the HCl is certainly effective, but its action is a rather indirect one, because the formation of $Ca(HCO_3)_2$ will not take place in the absence of CO_2, which is found by the interaction between HCl and $CaCO_3$. In fact the CO_2 in this case may have played an important role. For the solubility of CO_2 in a fluid such as blood or serum is by no means small, and consequently the amount of carbon dioxide contained as free CO_2 may be very large. Therefore that the CO_2-content observed in the acid experiment must have been composed partly of calcium bicarbonate and partly of free CO_2 dissolved becomes highly probable.

This relation can readily be made clear by extracting the free CO_2 alone from the sample to which no acid was added. But instead of such a test I have tried for the same purpose a small experiment in which 1 gm of powdered shell was added to 10 cc of acidified sea water (pH 1.20), and shaken vigorously. After standing at 25°C for 12 hours the water was found to contain 45.17 vol% of total CO_2, and of which 26.03 vol%, i. e. 50% of total CO_2, was found to be the free CO_2 dissolved. From these results just given, and moreover taking into consideration the small solubility of $Ca(HCO_3)_2$, I am inclined to maintain that the increase of CO_2 in the internal medium as well as in the external medium (as can be seen in the breathing water of

susceptibility experiment) may be attributed rather to the free CO_2 than to the $Ca(HCO_3)_2$.

Turning now to the alkali experiment, the speed of the change of pH has been found to be almost similar to that of the acid experiment, concerning both the blood and the pericardial fluid. The increase of the pH in this case may probably be due to the increase of OH ion, which has penetrated from breathing water to the internal fluids. The fact that the increase of OH ion occurs equally rapidly with the increase of H ion may be ascribed to the great adsorbability of these ions, when the traversing mechanism of these ions has been assumed as according to the adsorption phenomena, as TAYLOR[?] maintains in his explanation of the penetration of HCl through the cell membrane.

In Exp. I and Exp. II the pH of internal medium increased almost regulary during 24 hours of the experiment. Whereas in the Exp. III and in the experiment of susceptibility in which the experiment was conducted under severe alkalinity and continued as long as 48 hours, the change of the pH was not so regular as in the former cases. In fact some individuals showed a permanent rise in pH, while in the other the change was almost inappreciable or none. These irregular results can be taken as the peculiar effect of the severe alkalinity of breathing water. However from the result of Experiment II it can also be assumed that even the individuals (e. g. No. of Table VIII and No. of Table IX) which maintained their pH unaltered might have once increased their pH at the beginning of the experiment. If this was the case, the OH ion which once increased in the internal medium must have been excreted by some physiological process, and further the penetration of OH ion from breathing water must have ceased after that time. The excretion of excessive OH ion may probably have been carried by the kidney, while the faculty of preventing the inversion of OH ion may be accounted for as according to the change of the character of the gill membrane due to the severe alkalinity of breathing water.

The question why some individuals can regulate the pH while others cannot, or the question as to on what mechanism depends the regulation of permiability of gill membrane against OH ion cannot be answered as yet.

A special finding resulting from the alkali experiment revealed the

fact that the blood alkali behaved in a quite different manner as compared with that of the acid experiment. In the alkali experiment the CO_2-content showed no distinct change, notwithstanding that the pH of the blood and pericardial fluid showed a distinct change. The rise of the pH then apparently is not due to the formation of sodium bicarbonate in the blood, but due to a mere increase of OH ion which invaded from the breathing water without accompanying the sodium ion, because the CO_2-content must have been increased if the molecules of NaOH or both the anion and the cation of this salt penetrated simultaneously into the blood, as they would produce sodium bicarbonate or carbonate according to the following equations.

$$NaOH + H_2CO_3 = NaHCO_3 + H_2O$$
$$2NaOH + H_2CO_3 = Na_2CO_3 + 2H_2O$$
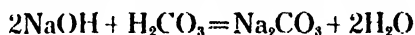
But any alkali the production of which is demanded by the above equations was not detected. It seems therefore reasonable to suppose that the high alkalinity of breathing water changed the structure of the gill membrane so as not to allow the NaOH molecule or Na ion to permeate. I may add also that it will not be unreasonable to presume that the OH ion alone has penetrated the gill membrane leaving the cation outside, though such would immediately build up an opposing electrostatic potential unless there might occur some compensation process at the same time. Because this compensation, I think, will be satisfied by the outward shifting of anion HCO_3 which is yielded by a dissociation of H_2CO_3 in the blood.

As to the air exposing test, the decrease of pH may be ascribed to the production of the CO_2 in the blood. The increase of CO_2-content observed in this experiment may undoubtedly be associated with the dissolved shell carbonate as is believed by COLLIP[4]. He explained the increase of CO_2-content by an equation cited in the preceeding page and it is most probably applicable to the case of the oyster.

COLLIP[4] suggested that *Mya arenaria* is a facultative anerobic organism by assuring that this animal yields the carbon dioxide in boiled sea water as well as when it was exposed to the atmospheric air. I think this may also hold good with the case of the oyster.

In my air exposing experiment the CO_2-content of the blood rose

from 4.35 to 40.19 vol% in the course of one week, showing an increase of 35.84 vol%. So that the increase of 35.84 vol% may be taken roughly as the product of anerobic respiration. If, therefore, this CO_2 was assumed to be evolved from the combustion of a carbo-hydrate such as glycogen, and if this assumption turns out to be true, then the same amount of oxygen must also be combusted. Conse-quently, if the quantity of the blood of an oyster is assumed to be 20 cc., then the CO_2 produced in the blood must be nearly 7.2 cc.. This assumption leads us to conclude that the amount of oxygen consumed in a week must also be 7.2 cc., or equivalent to the amount of oxygen contained in about 1 litre of sea water. Since there are no other resources to supply such a greater quantity of oxygen we are forced to conclude that it was supplied by the anerobic process.

I think it is least probable that the oyster respires aerobically through the mantle surface, though it sometimes opens the shell in the atmospheric air. I have as yet no data sufficient to discuss this question, so it is reserved to the future.

Finally I wish again to emphasize the facts concerning the suscepti-bility of the oysters to the change of experimental media. The fact that the critical hydrogen ion concentration of blood for the survival of the oyster has been found to be pH 5.00 and the pH of 4.80 or even 4.90 is fatal, seems to be highly significant, because the isoelectric point of serum protein of the oyster must possibly be in the neigh-bourhood of these pH. According to TADOKORO and WATANABE[9] the isoelectric point of blood protein of *Caudina chilensis* (an echino-dermata) lies between pH 4.78 and 4.90 which agreed, though perhaps accidentally, with the value just mentioned. At the isoelectric point the blood will show much different physical properties as compared with the case in the normal pH. For instance the osmotic pressure and viscosity will be minimized and the charge of the blood cell will be changed. Therefore it is presumed that the blood of the oyster would have altered its properties in a great degree near the death point.

SUMMARY.

(1) Data are given to show the normal pH and corresponding CO_2-content of the blood and the pericardial fluid of the oyster, and

moreover to determine the threshold pH concentration, which kills the oyster within 48 hours.

(2) Data are given to show the varied responses of the blood and pericardial fluid of the oyster to the pH change of breathing water, and further to show the respose to the exposure to the atmospheric air.

(3) The normal pH has been found to be 7.24 in blood, and 7.16 and 7.11 in pericardial and body fluid respectively. The CO_2-content was found to be 4.22 vol% and 4.00 vol% in blood and pericardial fluid respectively.

(4) The pH of the blood and the pericardial fluid was submitted to a seasonal change, decreasing in early summer and increasing in early winter.

(5) The blood, pericardial fluid, and body fluid show no appreciable protein error in determining the pH by means of the indicator method.

(6) The relation between the pH and the CO_2 vol% with respect to the blood and pericardial fluid was found to be as follows.

$$CO_2 \ vol\% = 1.87 \ pH-9.49 \ldots \text{Blood.}$$

$$CO_2 \ vol\% = 1.31 \ pH-6.49 \ldots \text{Pericardial fluid.}$$

(7) The pH of the blood and pericardial fluid of the oyster rapidly decreases when it is subjected to the respiration in the water of high acidity. The lower limit of the decrease of pH ' in vivo ' has been found to be pH 5.40. This decrease of pH appears to be due to the penetration of acid from breathing water into the body.

(8) The CO_2-content of the blood and pericardial fluid of the oyster gradually increases, when it respires in the water of high acidity. The maximum limit of this increase ' in vivo ' has been found to be 55.69 vol%. This increase of CO_2-content is considered to be due partly to the formation of calcium bicarbonate and partly to the free CO_2 dissolved in the blood and the pericardial fluid.

(9) The pH of the blood and the pericardial fluid of the oyster rapidly increase when the animal is submerged in the breathing water of high alkalinity. The upper limit of this pH increase ' in vivo ' has been found to be pH 8.45. This increase of pH is surely due to the penetration of OH ion from the breathing water.

(10) The CO_2-content of the blood and the pericardial fluid of the

oyster remains unaffected by the increase of blood pH. This was probably due to the selective permeability of plasma membrane, which enables the OH ion alone to permeate, because if Na ion or NaOH molecules penetrate into the blood the CO$_2$-content must increase, as they will combine with the CO$_2$ evolved from tissue.

(11) The CO$_2$-content of the blood and the pericardial fluid of the oyster increases and the pH of these fluids decrease when the animal is exposed in the atmospheric air. The former increase was probably due to the formation of calcium bicarbonate in the blood, and the latter decrease to the increase of CO$_2$ in the blood.

(12) The critical concentration of low blood pH which kills the oyster within 48 hours has been determined to be in the neighbourhood of pH 5.00.

(13) Susceptibility of oyster to high alkalinity (n/100 NaOH) is greater than to the high acidity (pH 1.20). In the former case the oyster survives for 48 hours while in the latter case they mostly die within 48 hours.

November, 1928.

LITERATURE CITED.

1) TAKATSUKI, S., 1927. Physiological studies on the rhythmical heart pulsation in the oyster, *Ostraea circumpicta* PILS. Science Rep., Tôhoku Imp. Univ., 4th Series, Vol. II, No. 3, pp. 301–324.

2) KOKUBO, S., 1927. On the hydrogen ion concentration and the CO$_2$-gas content and capacity of fish blood. Science Rep., Tôhoku Imp. Univ., 4th Series, Vol. II, No. 4, pp. 325–359.

3) AUSTIN, J. H. and CULLEN, G. E., 1926. Hydrogen ion concentration of the blood in health and desease. Medicine monograph, VIII.

4) COLLIP, J. B., 1921. Studies of molluscan coelomic fluid. Jour. Biol. Chem., Vol. XLV, pp. 23–49.

5) COLLIP, J. B., 1920. The alkali reserve of marine fish and invertebrates. Jour. Biol. Chem., Vol. XLIV, p. 329.

6) TAYLOR, N. W., 1928. Acid penetration into living tissues. Jour. Gen. Physiol., Vol. XI, No. 3. pp. 207–219.

7) ELIZABETH, H. and others., 1927. The protein error in estimating the pH with neutral red and phenol red. Biochem. Jour. Vol. 21, pp. 356–361.

8) TADOKORO, T. and WATANABE, S., 1928. Chemical studies on sex difference of blood protein in *Caudina chilensis* MÜLL. Science Rep., Tôhoku Imp. Univ., 4th series, Vol. III, No. 4, Fasc. 1, pp. 535–545.

The Power of the Adductor Muscle of the Oyster, *Ostraea circumpicta* Pils.

By

Tadashi Tamura.

(The Marine Biological Station of the Tôhoku Imperial University,
Asamushi, Aomori Ken, Japan.)

1. Introduction.

It is a matter of common knowledge that in order to open the shells by mere pulling against the contracting power of the adductor muscle requires considerable strength. Many physiologists have focussed their attention on this unique phenomenon for a long time.

Formerly Plateau (Regnard 1891, and Flattely and Walton 1922,) studied the power of the adductor muscle of several bivalves, and found that the number of gms. required per cm² of the section area of adductor muscle in order to overcome the closing power is enormous, and also found a wide range of difference among many species of Lamellibranchia in this regard. Plateau, however, limited his observation to determination of the absolute power of the adductor muscle, and therefore the question of the durability against the maximum weight was untouched. The present investigation was carried out to determine not only the power which was needed to tear off the adductor muscle of the oyster and other common bivalves, but has been extended to the observation of spontaneous shell movement, with or without loading with weight, and also the estimation of the power of the ligament against the natural closing power. The present experiment was carried out during the summer months of 1928 at the Asamushi Marine Biological Station. The oyster, *Ostraea circumpicta* Pils, which is found in abundance on the rocky tidal shore in the vicinity of our station, and the other common bivalves used, were collected at the same locality.

2. Method.

For determining the strength of the adductor muscle of the oyster the ventral shell was tightly fixed with a strong wire along the under side of an iron bar which was attached to an iron stand. In order to pull the adductor muscle vertically by a hung weight, a small hole was made on the dorsal shell near its margin, and through this hole the weight was hung by means of strong wire. The power of the muscle was estimated in terms of the weight which was necessary to pull the shells apart (Fig. 1). We assumed that the adductor muscle is an elastic body, and the amount of work W, done by the adductor muscle against loaded weight (w) was calculated by the following formula.
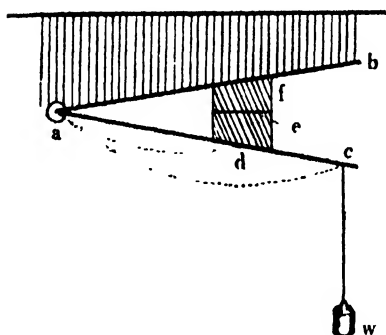
Fig. 1.

$$W = \frac{ac}{ad} w \ldots \ldots \ldots (1)$$

where ad = distance in centimeters from ligament to the center of adductor muscle.

ac = distance in centimeters from ligament to the point where the weight hangs.

w = hung weight in gram.

The section area cm² of the adductor muscle (e) was measured by using the planimeter. Finally the power of the adductor muscle per cm² of section area was calculated by the formula (2).

$$\frac{W}{e} = \text{gms. per cm}^2 \text{ section area} \ldots \ldots (2)$$

In order to observe the shell movement of the oyster under a loaded condition the apparatus equipped as aforementioned was dipped into a jar which was filled with running sea-water. The shell movement was recorded by the usual kymographic method, attaching a fine wire to the right shell, which was connected to a lever (Fig. 2).
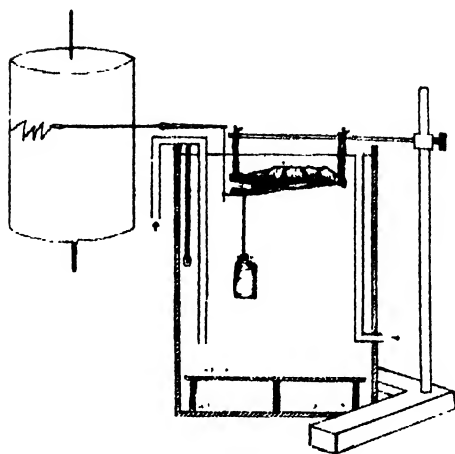
Fig. 2.

3. EXPERIMENTS.

Exp. 1. The opening and closing movement of the oyster under the normal condition.

Under the normal condition the oyster makes an opening and closing movement. Five oysters the body weights of which ranged from 430 to 520 gms. were submitted to the experiment as soon as these were collected. The observations on the shell movement were made during a period of hours under almost similar conditions. The results obtained are given in Table 1 (p. 272).

As will be seen from Table 1, two of the specimens employed showed the spontaneous movement of the shells for 24 hours continuously, and in the other two specimens the shell movements were noted for the first 19 hours and the first 5 hours respectively, after which the movement stopped till the end of 24 hours, while the remaining one showed no movement at all during the 24 hours. From the records obtained from the above list the time required to complete a spontaneous shell movement, opening and closing, was found to be from 3 m. 2 sec. to 11 m. 15 sec., and the amplitude of the shell opening ranged from 2.8 mm. to 7.0 mm. It is clear from the present results that within the normal oysters, or even with the same individual, the period of the shell movement as well as the amplitude of opening vary widely.

NELSON (1921) stated concerning the shell movement of the American oyster that it is influenced by the environmental factors such as day and night, tide, density, turbidity, temperature, and the number of food organisms.

According to the same investigator (1921), when too many food organisms appeared in the sea the shell movement ceased for a while.

KELLOG (1910) stated that when the surrounding medium is contaminated, the oyster defends against the invasion of mud or other substances into the body by closing the shell tightly.

Although I have also paid attention to the factors mentioned by NELSON, I have been thus far unable definitely to correlate the wide variability of shell movement with any of the factors mentioned above, except that an unusually wider opening of the shell than the normal width of the opening occurs once a while, which may be regarded as similar to the gill cleaning movement usually shown in fishes.

PAWLOW (1885) studied the opening and closing shell movement of *Anodonta* from the view point of nervous system and muscle physiology.

From the kymographic records (Fig. 1, 2, and 3 in Plate XII) we notice that the oyster opens the shell very slowly and sometimes even little by little, showing numerous steps. However, as soon as the opening reaches the maximum, the shell is closed usually quickly, though in some cases the shell remains widely opened for a while. The closing movement is usually instantaneous, contrary to the very slow opening movement. Although the majority of the oysters show alternative movements of opening and closing, yet in some the shell remains either in an opened or closed state for a considerable length of time.

MARCEAU (1905), who examined the resistance power of the adductor muscle of *Pecten maximum* and of *Mactra glauca,* observed that the adductor muscle of Acephales consists of two kinds of muscle fibre, and that the functions of the respective muscles also differ.

According to UEXKÜLL (1912) who studied the adductor muscle of *Pecten* and of sea-urchin, the adductor muscle of *Pecten* consists of a large round transparent part consisting of cross-striated fibres and a small opaque part formed of smooth fibres. According to their functions, he named the former muscle " Bewegung Muskel " and the latter muscle " Sperrung Muskel ". BAYLISS (538 p.) called the former " motor muscle " and the latter " catch muscle ".

In the present investigation it appeared to the present writer reasonable to ascribe the shell closing movement to the motor muscle, and the graditional movement to the catch muscle, as will be shown later.

Exp. 2. The shell movement under a loaded condition, and the time needed until the adductor muscle is torn off by a hung weight.

Fifteen oysters were tested under sea water, loaded with varying weights of 2 Kgs., 5 Kgs., 10 Kgs., 15 Kgs., 20 Kgs., 25 Kgs. and 30 Kgs. The details of the shell movement as affected by the loaded weights were recorded by the kymographic method. The results are given in Table 2 (pp. 272–273).

In the two oysters loaded with 2 Kgs. each adductor muscle withstood the weight for 5 days 10 hours and 1 day 6 hours respectively before being torn off. The shell movement (No. 6 Fig. 4 in Plate XII) was almost similar to that of the normal oyster unloaded. The frequency of the movement in the specimen No. 6 was 100 times in 24 hours, showing the time required for opening and closing was 3 m. $3\frac{3}{50}$ sec. in average, while in No. 7 it was 120 times in 24 hours, or an average time for each complete movement of 12 m.

Three oysters were loaded with 5 Kgs., and number of hours needed before the adductor muscle was torn off was observed in each. The results were 15 hours in No. 8, 20 hours in both No. 9 and No. 10. The shell movement (Fig. 5 in Plate XII) was almost similar to that of the 2 Kgs. loaded oyster, or in other words the movement was almost as normal as that shown by the unloaded oyster.

When a 10 Kgs. weight was hung on, the oyster showed few shell movements (Fig. 6 in Plate XII), and the adductor muscle was torn off after 2 hours 30 minutes in oyster No. 11, while in specimen No. 12 the muscle only withstood the strain for 7 minutes. This oyster did not move the shell at all.

If weights of over 15 Kgs. were loaded, the adductor muscle withstood for a very short time and in no instances were the shell movements observed.

When heavy weights were loaded the shell gradually opened, and the adductor muscle was torn off within 1 to 15 minutes.

The relation between the weights loaded and the time required

to tear off the adductor muscle found in the present experiment is
as follows :

Weights loaded.	Time required to tear off the adductor muscle.
2 Kgs.	1 day 6 hours to 5 days 10 hours.
5 Kgs.	15 hours to 20 hours.
10 Kgs.	7 minutes to 2 hours 30 minutes.
15 Kgs.	2 minutes to 15 minutes.
20 Kgs.	45 seconds to 3 minutes.
25 Kgs.	10 minutes.
30 Kgs.	1 minute.

The variations shown in the above table may be due to any one of the following factors, or all combined : size of the oyster itself, the difference in the area of the adductor muscle, and also to the physiological state of the oyster at the time of experiment. The relation between the weights acted on the adductor muscle per cm^2 section area and their endurance in times of hours required to tear off the muscles is shown in Fig. 3.

Exp. 3. Threshold weight for tearing off the adductor muscle of the oyster instantaneously.

In this experiment the threshold weight which if loaded tears off the adductor muscle at once was determined, and further the work (W) done by the adductor muscle, and the weight acting on per cm^2 of the section area of adductor muscle for loading, were also calculated. The amount of work (W) done by a unit area cm^2 of the adductor muscle was
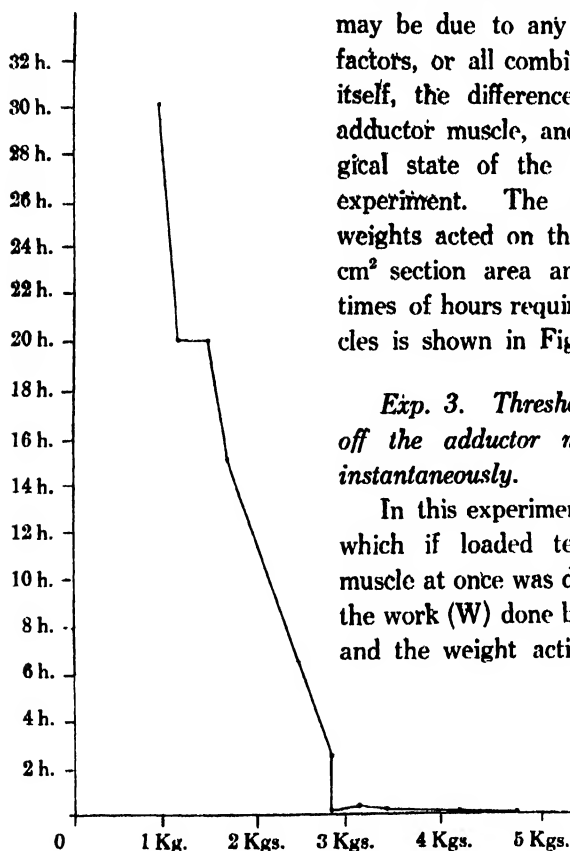
Fig. 3.   Ordinate — Duration of Experiment.
Abscissa — Power acting per cm^2 section
area of adductor muscle.

calculated by the formula 2. In order to ascertain the weights which acted on per cm^2 of adductor muscle, i. e., the muscle power, the following four lots of oysters were chosen according to their size, in as much as the muscle power may be proportional with the size of the animal. The weights of five oysters in each lot were as follows:

lot 1. 62 gms. to 100 gms.   lot 2. 200 gms. to 240 gms.
lot 3. 305 gms. to 366 gms.   lot 4. 518 gms. to 775 gms.

The results obtained from these twenty oysters represented by the four lots are given in Table 3 (p. 274).

The results show that the weights required in tearing off the adductor muscle instantaneously varies considerably according to individuals. For instance, No. 22 required 5 Kgs. while No. 38 required as much 40 Kgs. for this purpose. However, when the weights required are distributed per cm^2 of the section area of adductor muscle, then the unit area of the muscle of No. 22 requires 6900 gms. and that of No. 38 requires 7726 gms., showing that the difference of loaded weights shown by the different oysters was due to the corresponding differences in both the size of oyster and the area of adductor muscle.

From Table 3 we find that the power of the adductor muscle per cm^2 section varies considerably according to the individual, ranging between 5047 gms. (No. 24) and 10971 gms. (No. 34). We also notice the fact that the smaller individuals, such as in lot 1, give a smaller value, and conversely the larger oysters in lot 3 give larger values of mean muscle power, indicating in general that the power of the adductor muscle is stronger in larger specimens than in the smaller ones.

Lot.	Body weight (gms.)	Hung weight (Kg.)	(W) weight acting on the add. muscle (Kg.)	Area of the add. muscle (cm^2)	W per cm^2 section area of add. muscle (gms.)	Mean W per cm^2 section area (gms.)
1.	62–100.	5.0–11.0	6.9–17.8	1.0–2.3	5047– 8571	6789
2.	200–240.	9.0 15.0	13.7–25.8	2.3–4.1	5708– 8120	6975
3.	305–366.	14.0–25.0	22.9–38.4	3.1 4.9	6591 –10971	8289
4.	518–775.	32.0–40.0	41.5–56.4	5.0–7.3	7729– 9145	8637

From Table 3 the following mean values are obtainable:

An oyster of which the tissue weight is 28.1 gms. can withstand 7880 gms. per unit area of its adductor muscle, and the work done by the adductor muscle is 31300 gms., corresponding to 1110 times the tissue weight.

Exp. 4.   The power of the adductor muscle under narcotized condition.

The relative power of the adductor muscle of the oyster narcotized with menthol or chloroform, compared with that of normal oyster. The results obtained from five specimens are given in Table 4 (p. 274).

That the power of the muscle was weakened when the oyster was narcotized can readily be recognized. The muscle power estimated ranged from 2225 gms. to 5894 gms., and the mean value was estimated to be 4734 gms. per cm^2 against 7880 grams of the normal mean.

DE BUISSON (1927) studied the influence of the acid and alkali upon the muscle of *Anodonta* and reached the conclusion that the power of the muscle of *Anodonta* becomes stronger by HCl and becomes weaker by NaHCO$_3$, but the effect of narcotics was not observed.

Exp. 5.   The power of the adductor muscle of other common bivalves.

The power of the adductor muscle of several common bivalves was tested.

a) Anadara inflata REEVE.   (Akagai).

This bivalve is provided with two adductor muscles of nearly equal size, like those of *Anodonta*, thus differing from the oyster which has but one muscle.   So that, in this case, the factor " ad " in formula 1 represents the distance from the ligament to the middle point of a line which connects the two adductor muscles.   According to this formula the power of the muscle was calculated, based on the data obtained from five specimens, and the results are given in Table 5 (p. 275).

The total body weight of the *Anadara* varies from 288 to 650 gms.   5.0 to 11.0 Kgs. were loaded.   The weight acting on the adductor muscle was calculated, and the results were 21.2 to 44.0 Kgs. The power of the adductor muscle per cm^2 section area was 5116 gms. to 8028 gms., showing a mean value of 6374 gms.   A comparison between the mean tissue weight and the mean W which acted on the adductor muscle was found to be 185 gms.: 31500 gms. (=1 : 170). The factor W becomes, in the present case, disproportionately large when compared with its hung weight, owing to the very small dis-

tance in this species from the ligament to the adductor muscle.

b) Anodonta lauta MARTEN. var. (Dobugai).

The results obtained from seven tests conducted on seven different individuals are shown in Table 6 (p. 275).

The body weight ranges from 91 gms. to 166 gms. The weight needed in tearing off the adductor muscle instantly after loading was 2.3 Kgs. to 3.2 Kgs. The work done, W, calculated by formula 1, was found to be 8.2 Kgs. to 10.4 Kgs. The power of the adductor muscle per cm^2 section area was 2928 gms. The ratio of the average tissue weight to the average W was 38 gms.: 9100 gms. (=1 : 239.4). Consequently the W which acted on the adductor muscle is consideraly large, notwithstanding that the hung weight was small.

c) Mytilus crassitesta LISCHKE. (Igai).

The results obtained from seven tests are given in Table 7 (p. 275).

The body weight of the specimens used varied from 92 gms. to 195 gms. The weights required in tearing off the adductor muscle instantly after loading were 18 Kgs. in minimum and 23 Kgs. in maximum. The W calculated by formula 1 was found to be 14.8 Kgs. to 26.3 Kgs. The power of the adductor muscle per cm^2 section area was estimated to be 3895 gms. to 6877 gms., and its mean weight 5586 gms. The ratio of average tissue weight to average W can be shown as 28.4 gms. : 18900 gms. (=1 : 665).

d) Modiolus barbatus LINNÉ. (Hibarigai).

The present species much resembles *Mytilus* in its form as well as in its habitat. The data were obtained from eight specimens and are tabulated in Table 8 (p. 276).

The estimated weight of the entire body ranges from 70 gms. to 130 gms. The weights used were 5 Kgs. to 9 Kgs. The work W calculated by formula 1 was 6.7 Kgs. to 10.6 Kgs. The power of the adductor muscle per cm^2 section area was calculated to be 2466 gms. to 3533 gms., and its average as 2809 gms. The ratio of the mean tissue weight to the mean W were 13.4 gms.: 8300 gms. (= 1 : 619).

e) Pecten yessoensis JAY. (Hotategai).

The results obtained from five individuals were shown in Table 9 (p. 276).

The body weights were 220 gms. 'to 289 gms. The hung weights were 4.0 Kgs. to 6.7 Kgs. The work W calculated by formula 1 was

7.1 Kgs. to 11.0 Kgs. The power of the adductor muscle per cm^2 section area were 293 gms. to 785 gms., and its mean was 445 gms. The ratio of the average tissue weight to the mean W was 105 gms.: 8800 gms. ($=1:84$).

f) Chlamys senatorius REEVE. (Akasaragai).

The results obtained from five individuals are given in Table 10 (p. 276).

The body weights of the specimens used were 45 gms. to 105 gms. The hung weights were 2.1 Kgs. to 3.0 Kgs. The work W calculated by formula 1 were 2.6 Kgs. to 4.8 Kgs. The power of the adductor muscle per cm^2 section area was calculated to be 900 gms. to 1966 gms., and its mean was 1272 gms. The ratio of the mean tissue weight to the mean W was found to be as follows: — 22.6 gms.: 3560 gms. ($=1:175$).

Exp. 6. The power of the ligament of the oyster and of the other common bivalves.

The ligament of the Lamellibranchia acts to open the shells in opposition to the closing power of the adductor muscle. The opening power of the ligaments against the closing power of the muscle was estimated with several bivalves, including the oyster. The method adopted to estimate this power of ligament (W) was as follows: the oyster shell opens widely just as soon as the adductor muscle is severed completely, and the weights (w) needed to close the shells entirely have been determined. The method of calculation used was the same as that used in the case of the adductor muscle.

$$W = \frac{b}{a}\, w.$$

where   W = power acting on the adductor muscle by weight w.

w = weight needed to close the shells entirely.

a = distance from ligament to adductor muscle.

b = distance from ligament to a point where weight was added.

From the data given in the Tables 11 to 17 (pp. 277–279) following results can be obtained.

Species.	No. of animals tested.	Size (gms.)	Weight added (gms.)	(W) power of the lig. acting on add. mus. (gms.)	Mean (W) (gms.)	Lig. power acting per cm² (gms.)
Ostraea circumpicta Pils.	5	328–775	500–3200	769–3500	2176	347
Anadara inflata Reeve.	5	350–375	50– 190	200– 570	338	65
Anodonta lauta Marten. var.	3	80–110	180	603– 780	676	282
Mytilus crassitesta Lischke.	5	50–140	600 1300	1000–2080	1414	416
Modiolus barbatus Linné.	5	48–130	400– 700	421– 622	563	188
Pecten yessoensis Jay.	5	220–289	125– 165	214– 268	276	14
Chlamys senatorius nobilis Reeve.	5	58–145	50– 110	91– 168	146	40

It will be seen from the above that the power of the ligament differs according to the species as well as to the individual. The individual difference was most marked in the oyster. As the ligament acts oppositely to the adductor muscle the ligament power must be added to the power of the muscle already found. Comparison of the ratio between mean tissue weight and the mean W showed the following order according to the species:

Ostraea > *Mytilus* > *Modiolus* > *Anodonta* > *Anadara* >
Chlamys > *Pecten*.

By a glance at this order, one will notice the fact that the power of the adductor muscle shows some interrelation to the habitat; that is, the inhabitants of shallow water which is disturbed by the waves and tides, possess stronger muscle than those living in the deep water. Now comparing the power of the ligament, we find the following order according to the species:

Ostraea > *Mytilus* > *Modiolus* > *Anodonta* > *Anadara* >
Pecten > *Chlamys*.

This order, according to the power of the ligament, agrees very well with the order already given according to the power of the adductor muscle; showing that the bivalves possessing adductor muscles of greater power possess correspondingly powerful ligaments. Stoutness of the shell also develops in concert with the muscle power of the shell. As to the question whether both the powerful adductor muscles and ligaments are associated with better developed or relatively thicker shells, we found the following relation:

Ostraea 6 mm., *Anadara* 4 mm., *Mytilus* 3 mm., *Modiolus* 1.7 mm.,

Pecten 1.5 mm., *Anodonta* 1.2 mm., *Chlamys* 1.2 mm.

The correlation between the powerful muscles and shell thickness is small, and therefore we may assume that the shell thickness must be the result of some other factors which are not yet determined.

SUMMARY.

1.   The shell movement of the oyster under normal condition much differs according to the individual.

2.   The adductor muscle of the oyster consists of two different parts functionally different, the motor muscle and the catch muscle.

3.   The oyster makes normal spontaneous shell movements in the sea water when loaded with 5 Kgs. but when loaded with 15 Kgs. or more, the shell movement is not exhibited at all.

5.   The power of the adductor muscle per cm² section area of the oyster differs according to the dimension of the oyster, and is weaker with smaller ones and stronger with larger.

5.   Narcotized oysters show marked diminution of the power of the adductor muscle.

6.   Ratio between the total body weight and its tissue weight, between tissue weight and the adductor muscle weight, and between the adductor muscle weight and the section area of adductor muscle differs considerably according to the individual.

7.   The power of the adductor muscle of several other species of common bivalves was determined.

8.   The power of the adductor muscle of several bivalves seems to show that the inhabitants of shallow water possess greater power than those of deep water.

9.   The power of the ligament of several bivalves is proportional to the power of the adductor muscle with the species so far tested.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.
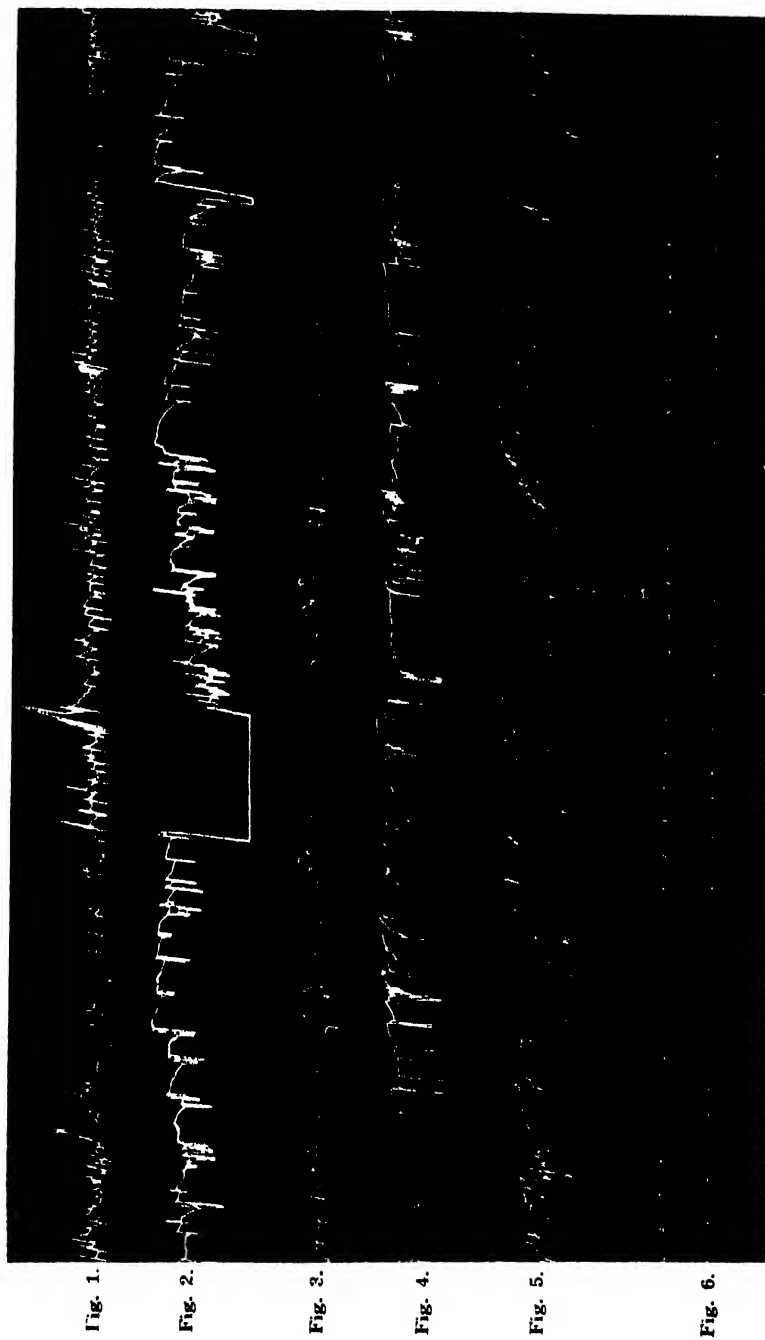
T. TAMURA : Adductor Muscle of Oyster.

LITERATURE CITED.

1) REGNARD, PAUL. 1891. Recherchs expérimentales sur les conditions physique de la vie dans les eaux. (locomotion).

2) FLATTELY, F. W. and C. L. WALTON. 1922. The biology of the seashore. p. 110.

3) NELSON, THURLOW C. 1920. Report on the department of biology of the New Jersey Agricul. College Exper. Station. pp. 335.

4) PAWLOW, I. P. 1885. Wie die Muschel ihre Schale öffnet. Pflügers Arch., Bd. 37, S. 6-31.

5) MARCEAU, M. F. 1905. Sur la production de travail méchanique par les muscles adducteurs des acéphales. C. R. de l'acad., T. 141, pp. 278.

6) BUDDENBROCK, W. 1925. Grundriss der vergleichenden Physiologie. 2. Teil, S. 435.

7) BAYLISS, W. M. 1920. Principles of general physiology. p. 538.

8) KELLOG, J. L. 1910. Shell fish industry. pp. 55.

9) DU BUISSON, M. 1928. Influence de HCl et NaHCO₃ sur le degré de tension des muscles adducteurs des valves des anodontes. Ber. ü. d. ges. Biol. Abt. B, Bd. 45, Heft 11/12.

EXPLANATION OF PLATE XII.

Fig. 1. Shell movement under normal condition. 1 scale — 15 minutes. (Experiment No. 3).

Fig. 2. Shell movement under normal condition. 1 scale — 15 minutes. (Experiment No. 4).

Fig. 3. Shell movement under normal condition. 1 scale = 15 minutes. (Experiment No. 5).

Fig. 4. Shell movement when hung with 2 Kgs. weight. 1 scale — 15 minutes. (Experiment No. 6).

Fig. 5. Shell movement when hung with 5 Kgs. weight. 1 scale = 15 minutes. (Experiment No. 10).

Fig. 6. Shell movement when hung with 10 Kgs. weight. 1 scale — 5 minutes. (Experiment No. 11).

TABLE 1.

The opening and closing movement of the oyster under normal condition.

No.	Weight	Length	Breadth	Frequency of movement	Period	Amplitude	Date	Water temp.
	gms.	c. m.	c. m.			m. m.		
1	430	12.0	8.5	0 in 24 h.	—	—	Aug. 28	22.°C.
2	500	14.0	10.0	44 times in 5 h.	6 m. 48 $\frac{1}{11}$ s.	2.8	" "	22.°C.
3	480	13.0	8.5	375 times in 24 h.	3 m. 2 $\frac{10}{13}$ s.	7.0 (6 times) 3.0	Sept. 13	24.°C.
4	520	14.5	8.5	142 times in 19 h.	7 m. $\frac{12}{71}$ s.	7.0 (5 times) 4.0	" "	24.°C.
5	515	13.0	9.0	138 times in 24 h.	11 m. 15 s.	3.0	Aug. 28	22.°C.

TABLE 2.

The shell movement under a loaded condition, and the time needed until the adductor muscle is torn off by a hung weight.

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Distance from lig. to add. mus.	lig. to hung point	Hung weight	W	W per cm².	Duration	Shell movement	Period	Amplitude	Date	Water temp.
	gms.	c. m.	c. m.	gms.	cm².	c. m.	c. m.	kgs.	gms.	gms.				m. m.		
6	480	16.0	9.5	32.0	3.6	5.5	9.0	2	3272	935	30 h.	480 in 24 h.	3 m. 3¾ s.	6.5	Sept. 14th	24.°C.
7	450	15.0	8.5	25.0	4.9	7.5	11.0	2	2667	544	5 d. 10 h.	120 in 24 h.	12 m.	11.0 6.5	"	24.°C.
8	310	10.0	8.0	22.0	4.5	6.0	9.0	.5	7500	1666	15 h.	23 in 5 h.	13 m. 2 s.	3.0	Aug. 15th	22.°C.

TABLE 2. (Continued)

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Distance from lig. to add. mus.	Distance from lig. to hung point	Hung weight	W	W per cm.³	Duration	Shell movement	Period	Amplitude	Date	Water temp.
	gms.	c.m.	c.m.	gms.	cm.²	c.m.	c.m.	kgs.	gms.	gms.				m.m.		
9	610	15.0	11.0	60.0	6.4	7.0	10.5	5	7500	1115	20 h.	80 in 20 h.	15 m.	4.0	Sept. 3rd	24 °C.
10	370	14.5	10.0	28.0	5.8	6.5	10.5	5	8476	1461	20 h.	120 in 20 h.	10 m.	10.0 4.5	"	24½ °C.
11	595	17.0	9.0	52.0	5.7	7.5	12.0	10	16000	2807	2 h. 30 m.	50 in 2½ h.	3 m.	0.2	Aug. 10th	22 °C.
12	400	12.0	8.0	39.0	5.0	5.7	8.0	10	14038	2877	7 m.	—	—	—	"	22 °C.
13	466	16.5	7.5	48.0	4.5	6.6	8.5	15	10915	4225	2 m.	—	—	—	Aug. 19th	22 °C.
14	430	13.0	9.0	36.0	4.1	6.0	7.5	15	18750	3125	15 m.	—	—	—	Sept. 26th	23 °C.
15	400	12.0	7.0	30.0	2.5	6.5	9.0	20	27692	11076	45 s.	—	—	—	Aug. 21th	22 °C.
16	420	13.5	7.5	52.0	5.3	5.5	7.5	20	25454	4803	50 s.	—	—	—	Aug. 22th	22 °C.
17	460	14.0	8.0	39.0	5.2	7.2	10.0	20	25000	4807	1 m.	—	—	—	"	22 °C.
18	650	19.0	8.0	68.0	4.0	9.0	12.5	20	27777	6944	3 m.	—	—	—	Aug. 23th	22 °C.
19	750	20.0	11.0	78.0	10.3	8.0	11.0	25	34875	3367	10 m.	—	—	—	Aug. 21th	22 °C.
20	576	15.0	9.0	50.0	5.0	7.0	10.0	30	42857	8571	1 m.	—	—	—	Aug. 22th	22 °C.

TABLE 3.

Threshold weight for tearing off the adductor muscle
of the oyster in a moment.

No.	Weight	Length	Breadth	Tissue weight	Add. mus. weight	Area of add. mus.	Hung weight	Distance from lig. to add. mus.	Distance from lig. to hung point	W	W per cm²	W per cm² on each lots	Date
	gms.	c. m.	c. m.	gms.	gms.	cm²	kgs.	c. m.	c. m.	kgs.	gms.	gms.	
21	62	6.0	5.0	6.5	1.0	1.4	8.0	3.5	5.5	21.0	8571		Nov. 3
22	68	7.0	5.0	3.5	0.5	1.0	5.0	4.0	5.5	6.9	6900		,,
23	93	8.5	5.5	10.0	1.5	1.3	8.5	5.5	6.5	10.0	7692		,,
24	96	9.0	4.5	11.0	1.0	2.1	8.5	4.0	5.0	10.6	5043		,,
25	100	8.5	5.5	13.0	1.5	2.3	11.0	4.0	6.5	17.8	7739	6789	,,
26	230	11.0	6.0	13.5	2.5	2.3	12.0	4.5	6.5	17.3	7521		,,
27	240	11.0	7.0	27.5	2.4	2.4	9.0	5.0	7.6	13.7	5708		,,
28	235	11.0	7.5	24.0	2.8	3.0	15.0	4.5	6.5	21.7	5233		,,
29	200	12.0	7.0	15.0	3.2	4.1	14.5	5.2	8.0	25.8	6292		,,
30	220	12.0	7.0	18.0	3.5	2.5	13.5	5.0	7.5	20.3	8120	6975	,,
31	366	13.0	8.8	30.0	4.0	3.8	19.0	5.0	9.0	34.2	9000		Nov. 4
32	305	13.0	7.2	32.0	3.5	4.9	21.0	6.5	10.0	32.3	6591		,,
33	350	12.0	8.0	50.0	4.6	3.4	19.0	5.0	8.5	32.3	9500		,,
34	228	15.0	7.0	25.0	4.0	3.5	25.0	6.5	10.0	38.4	10981		,,
35	336	12.5	7.5	19.0	3.3	3.1	14.0	5.0	8.2	22.9	7384	8289	,,
36	575	16.5	11.5	52.5	6.3	5.3	32.0	8.0	12.0	48.0	9056		,,
37	618	18.0	9.5	47.3	10.6	5.3	35.0	8.0	11.5	50.3	9145		,,
38	775	18.0	11.0	60.0	6.8	7.3	40.0	8.5	12.0	56.4	7726		,,
39	518	16.0	10.5	48.0	7.5	5.1	32.0	7.0	10.0	45.7	8960		,,
40	545	16.0	10.5	58.0	7.1	5.0	38.0	7.5	9.5	41.5	8300	8637	,,
Average.				28.1						31.3	7880		

TABLE 4.

The power of tearing off the adductor muscle of the oyster in a
moment under narcotized condition.

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Hung weight	Distance from lig. to add. mus.	Distance from lig. to hung point	W	W per cm²	Narcotized	Date
	gms.	c. m.	c. m.	gms.	cm²	kgs.	c. m.	c. m.	kgs.	gms.		
41	320	10.0	6.5	25	4.4	15	4.7	7.0	24.2	5636	3 h. in chroloform	Aug. 24th
42	290	13.0	7.0	35	4.5	12	5.1	7.0	18.4	4090	5 h. in menthol	,,
43	400	12.0	9.0	35	5.6	22	6.1	9.0	32.4	5870	12 h. in ,,	,,
44	420	14.0	8.0	25	3.1	5	7.2	9.0	6.9	2225	5 h. in chroloform	Aug. 25th
45	570	16.0	8.0	40	5.6	21	7.3	11.2	33.0	5894	19 h. in menthol	,,
Average				33						4743		

TABLE 5.
Anadara inflata REEVE.

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Hung weight	Distance from lig. to add. mus.	Distance from lig. to hung point	W	W per cm²	Date
	gms.	c. m.	c. m.	gms.	cm²	kgs.	c. m.	c. m.	kgs.	gms.	Aug.
1	320	12.0	8.0	165	4.6	8.0	2.0	6.4	25.6	5563	22th
2	345	12.0	7.5	180	5.1	9.0	2.0	7.5	33.7	8023	,,
3	288	12.0	7.0	138	3.9	8.0	1.7	7.0	33.0	7871	,,
4	650	13.5	9.0	330	8.1	11.0	2.3	9.2	44.0	5116	,,
5	375	12.2	9.0	123	4.0	5.0	2.0	8.5	21.2	5300	,,
Average				185					31.5	6374	

TABLE 6.
Anodonta lauta MARTENS. var.

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Hung weight	Distance from lig. to add. mus.	Distance from lig. to hung point	W	W per cm²	Date
	gms.	c. m.	c. m.	gms.	cm²	kgs.	c. m.	c. m.	kgs.	gms.	Sept.
1	98	9.5	6.7	33	2.9	2.6	1.8	6.0	8.7	3000	21th
2	110	10.5	5.7	39	3.0	3.1	1.7	5.7	10.4	3466	,,
3	100	9.5	5.4	33	2.2	2.3	1.7	5.4	7.3	3318	,,
4	91	9.5	5.5	30	2.0	2.5	1.5	5.5	9.4	4550	,,
5	85	9.3	5.5	26	2.3	2.7	1.8	5.5	8.2	2928	,,
6	80	8.5	5.2	31	2.0	2.7	1.5	5.2	9.3	4650	,,
7	166	11.5	6.5	75	3.0	3.2	2.0	6.5	10.4	3466	,,
Average				38					9.1	3625	

TABLE 7.
Mytilus crassitesta LISCHKE.

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Hung weight	Distance from lig. to add. mus.	Distance from lig. to hung point	W	W per cm²	Date
	gms.	c. m.	c. m.	gms.	cm²	kgs.	c. m.	c. m.	kgs.	gms.	Sept.
1	105	10.0	5.0	21	3.0	21.0	4.3	3.7	18.5	6166	13th
2	195	12.5	6.0	48	3.8	23.0	5.2	6.0	26.3	6821	,,
3	92	10.0	5.0	22	3.8	18.0	4.5	3.0	14.8	3895	,,
4	130	11.0	5.8	28	3.8	22.0	5.2	4.4	17.7	4658	,,
5	127	11.0	5.5	30	3.6	22.0	5.5	4.5	18.0	5000	,,
6	108	10.5	5.2	25	3.6	20.0	5.0	4.5	18.0	5000	,,
7	92	11.0	5.2	25	3.1	19.0	5.0	4.5	19.1	6161	,,
Average				28.4					18.9	5386	

TABLE 8.
Modiolus barbatus LINNÉ.

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Hung weight	Distance from		W	W per cm²	Date
							lig. to add. mus.	lig. to hung point			
	gms.	c.m.	c.m.	gms.	cm²	kgs.	c.m.	c.m.	kgs.	gms.	Sept.
1	130	10.5	5.6	22.0	3.6	9.0	4.5	5.0	10.0	2777	26th
2	90	9.5	5.0	20.0	3.0	9.0	3.8	4.5	10.6	3533	,,
3	85	9.0	5.0	12.0	2.9	6.5	4.0	4.5	7.2	2482	,,
4	70	8.5	4.5	11.0	2.9	6.5	3.5	4.0	8.0	2758	,,
5	82	8.5	5.0	14.0	2.9	7.7	4.0	4.0	7.7	2655	,,
6	63	7.8	4.5	10.0	2.9	7.0	3.5	4.0	8.0	2758	,,
7	53	8.0	4.2	12.0	3.0	6.5	3.5	4.0	7.4	2466	,,
8	48	7.2	3.8	6.5	2.0	5.0	2.8	3.2	6.7	3049	,,
Average				13.4					8.3	2809	

TABLE 9.
Pecten gessoensis JAY.

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Hung weight	Distance from		W	W per cm²	Date
							lig. to add. mus.	lig. to hung point			
	gms.	c.m.	c.m.	gms.	cm²	kgs.	c.m.	c.m.	kgs.	gms.	Sept.
1	220	13.5	14.0	99	21.0	4.0	7.0	12.5	7.1	239	14th
2	289	13.0	14.0	140	24.5	6.7	7.5	12.5	11.0	785	,,
3	240	13.0	14.0	94	20.0	5.2	7.0	12.5	8.5	425	,,
4	235	13.0	14.5	110	21.4	5.0	7.5	12.5	8.2	368	,,
5	220	12.0	12.5	83	24.5	5.2	6.5	11.5	9.2	408	,,
Average				105					8.8	445	

TABLE 10.
Chlamys senatorius nobilis REEVE.

No.	Weight	Length	Breadth	Tissue weight	Area of add. mus.	Hung weight	Distance from		W	W per cm²	Date
							lig. to add. mus.	lig. to hung point			
	gms.	c.m.	c.m.	gms.	cm²	kgs.	c.m.	c.m.	kgs.	gms.	Aug.
1	65	7.0	6.0	25	2.8	3.0	4.0	6.5	4.8	1777	24th
2	105	7.5	6.5	30	4.5	2.7	5.0	7.5	4.0	900	,,
3	70	7.0	6.0	25	3.2	2.5	4.5	6.5	3.6	1125	,,
4	50	6.0	5.0	17	2.4	2.1	4.5	6.0	2.8	1191	,,
5	45	5.7	5.0	16	1.5	2.1	4.3	5.5	2.6	1966	,,
Average				22.6					3.5	1272	

TABLE 11.

Ostraea circumpicta PILS.

No.	Weight	Length	Breadth	Area of add. mus.	Added weight	lig. to add. mus.	lig. to added point	W	W per cm²	Date
	gms.	c. m.	c. m.	cm²	gms.	c. m.	c. m.	gms.	gms.	Aug.
1	450	13.0	8.5	4.9	1700	8.5	11.0	2200	473	22th
2	520	17.0	11.0	8.0	3200	9.0	10.0	3500	437	,,
3	328	15.0	7.0	3.5	500	6.5	10.0	769	118	,,
4	775	18.0	11.0	7.3	1500	8.5	12.0	1899	260	,,
5	456	16.0	12.0	5.6	2320	8.5	12.0	2513	448	,,
Average								2176	347	

TABLE 12.

Anadara inflata REEVE.

No.	Weight	Length	Breadth	Area of add. mus.	Added weight	lig. to add. mus.	lig. to added point	W	W per cm²	Date
	gms.	c. m.	c. m.	cm²		c. m.	c. m.	gms.	gms.	Aug.
1	375	12.2	9.0	4.0	50	2.0	8.0	200	50	22th
2	350	11.5	8.0	4.0	60	2.0	7.0	210	52	,,
3	420	12.2	8.5	5.8	190	2.0	6.0	570	98	,,
4	370	12.0	8.0	5.2	70	2.0	6.0	210	40	,,
5	390	12.0	8.0	5.4	150	2.0	6.0	450	83	,,
Average								338	64.6	

TABLE 13.

Anodonta lauta MARTENS. var.

No.	Weight	Length	Breadth	Area of add. mus.	Added weight	lig. to add. mus.	lig. to added point	W	W per cm²	Date
	gms.	c. m.	c, m.	cm²	gms.	c. m.	c. m.	gms.	gms.	Sept.
1	110	10.5	5.7	3.0	180	1.7	5.7	603	201	21th
2	92	10.0	6.0	2.5	180	1.5	5.4	648	259	,,
3	80	8.5	5.2	2.0	180	1.2	5.2	780	387	,,
Average								676	282	

TABLE 14.

Mytilus crassitesta LISCHKE.

No.	Weight	Length	Breadth	Area of add. mus.	Added weight	Distance from lig. to add. mus.	Distance from lig. to added point	W	W per cm²	Date
	gms.	c. m.	c. m.	cm²	gms.	c. m.	c. m.	gms.	gms.	Sept.
1	140	12.0	5.5	3.8	1300	5.0	8.0	2080	525	13th
2	110	9.5	5.0	3.6	700	4.0	7.0	1225	361	,,
3	50	7.0	4.0	2.4	650	3.2	5.0	1017	457	,,
4	110	11.0	5.3	3.5	1000	4.0	7.0	1750	485	,,
5	110	10.0	5.3	3.5	600	4.5	7.5	1000	285	,,
Average								1414	416	

TABLE 15.

Modiolus barbatus LINNÉ.

No.	Weight	Length	Breadth	Area of add. mus.	Added weight	Distance from lig. to add. mus.	Distance from lig. to added point	W	W per cm²	Date
	gms.	c. m.	c. m.	cm²	gms.	c. m.	c. m.	gms.	gms.	Sept.
1	130	10.5	5.5	3.6	700	4.5	4.0	622	182	26th
2	70	8.5	4.5	2.9	600	3.0	4.0	600	207	,,
3	82	8.5	5.0	2.9	600	4.0	4.0	600	207	,,
4	63	7.8	4.5	2.9	500	3.5	4.0	571	192	,,
5	48	7.2	3.8	3.0	400	2.8	3.2	421	140	,,
Average								563	183	

TABLE 16.

Pecten yessoensis JAY.

No.	Weight	Length	Breadth	Area of add. mus.	Added weight	Distance from lig. to add. mus.	Distance from lig. to added point	W	W per cm²	Date
	gms.	c. m.	c. m.	cm²	gms.	c. m.	c. m.	gms.	gms.	Sept.
1	220	13.5	14.0	21.0	165	7.0	12.0	240	11	14th
2	289	13.0	14.0	24.5	115	7.0	12.0	368	15	,,
3	240	13.0	14.0	20.0	125	7.0	12.0	214	11	,,
4	235	13.0	14.5	21.4	175	7.0	12.0	300	13	,,
5	220	12.0	12.5	24.5	165	7.0	11.0	259	11	,,
Average								276	14	

TABLE 17.

Chlamys senatorius nobilis REEVE.

No.	Weight	Length	Breadth	Area of add. mus.	Added weight	Distance from lig. to add. mus.	lig. to added point	W	W per cm²	Date
	gms.	c. m.	c. m.	cm²	gms.	c. m.	c. m.	gms.	gms.	Sept.
1	145	9.0	10.0	5.0	110	6.0	8.0	143	29	21th
2	72	6.8	7.2	3.2	100	3.5	6.0	168	52	,,
3	70	7.8	8.0	4.0	80	3.0	6.0	160	40	,,
4	58	6.8	7.5	3.0	50	3.0	5.5	91	30	.,
5	71	7.0	7.5	3.2	90	3.0	5.5	165	51	,,
Average								146	40	

Chemical Analysis on the Pericardial Fluid and the Blood of *Ostrea Circumpicta* Pils.*

By

MASAO KUMANO.

(Biological Institute, Tôhoku Imperial University, Sendai.)

The saline content of the pericardial fluid and blood was determined in regard to *Ostrea circumpicta* Pils. living in the neighbourhood of the Asamushi Marine Biological Station. The materials were collected during the month of July, 1928. The blood was collected from the heart directly by means of a syringe, and usually from 8 to 12 oysters were used for a single determination. The pericardial fluid was also collected from the same oysters which were used for the collection of blood.

In the analysis of the pericardial fluid and the blood the following methods were employed. Sodium was determined by the MICHAEL BÁLINT[1] method, potassium by the KRAMER and TISDALL[2] method, magnesium by the HAMMETT and ADAMS[3] method, chloride by the RUSZNYÀK[4] method and sulphate by the DENIS[5] method. For the determination of the calcium the KRAMER and TISDALL method as modified by CLARK and COLLIP[6] was used.

The percentage content of the water in pericardial fluid and in blood of *Ostrea circumpicta* Pils. was found to be as follows:

Pericardial fluid 96.38

Blood 96.26

The following results represent the content of each inorganic substance in 100 cc. The figures on pericardial fluid and blood are the average of the 11 estimations. The figures given in ( ) denote the corresponding values when the sodium was taken as 100.

	Pericardial fluid.	Blood.	Sea water[7]
Na	1.1099(100.00)	1.1960(100.00)	1.1634(100.00)
K	0.0354(3.19)	0.0362(3.03)	0.0357(3.07)

---

* A contribution from the Marine Biological Station, Asamushi, Aomori-Ken, and the Biological Institute, Tôhoku Imperial University, Sendai.

	Pericardial fluid.	Blood.	Sea water [7]
Ca	0.0416(3.75)	0.0425(3.55)	0.0431(3.70)
Mg	0.1303(11.74)	0.1530(12.79)	0.1373(11.80)
Cl	1.9520(175.90)	2.1060(176.09)	1.9428(166.99)
SO$_4$	0.2673(24.09)	0.2821(23.60)	0.2627(22.58)

It will be observed that both the blood and the pericardial fluid show the closest similarity to, if not being identical with, the sea water ; which leads me to suppose that the blood and the pericardial fluid have nearly the same saline composition as the water in which they live. As far as the analysis shows, the amount of inorganic matter contained in the blood is somewhat greater than that of the pericardial fluid, especially in the content of magnesium when sodium is taken as 100.

Recently OKAZAKI and KOIZUMI[7] determined the inorganic matter in the body fluid of *Caudina chilensis* and found the following relations :

Na   100; K   3.43; Ca   3.43; Mg   10.00; Cl   175.00.

We notice then that the body fluid of *Caudina* is essentially the sea water where they are living, and consequently it also very closely resembles that of the oysters studied by me. However, we notice a conspicuous difference between the blood of the oyster and the body fluid of *Caudina* with respect to the percentage content of magnesium. In the oyster blood the content of magnesium is 12.79% while that in the body fluid of *Caudina* is only 10%, and is less than that given by the pericardial fluid of the oyster, in which it is 11.74%.

Whether or not this difference shown above is a real one, or is due to difference in the methods used, needs further studies ; but at any rate we note this difference with interest, considering the fact that *Caudina* is sand dweller while the oyster lives in the open sea.

The follwing table shows the results of chemical analysis of the blood made by A. B. GRIFFITHS[8*,9*], sodium being taken as 100.

Species	Na	K	Ca	Mg	Cl	SO$_4$
Pecten	100	12.35	8.07	3.30	116.00	10.38
Mytilus edulis	100	12.22	8.17	3.44	116.46	10.22
Mya	100	12.41	7.80	3.41	114.70	10.09

As the table shows, the percentage composition of inorganic matter shown by the three species is very similar in each case, in spite of the fact these belong to widely different molluscan genera. However these data differ considerably from those of the present writer on the oyster.

In 1920 MYERS determined some inorganic constituents in the blood of *Schizotherus Nuttalli* and found 3.19 g. of NaCl and 197 mg. of CaO and also in *Saxidomus Nuttalli* found 307 mg. of CaO. These values just quoted are compared with mine, taking sodium as 100 in all cases, and are given below.

Species	Na	Cl	Ca
Schizotherus Nuttalli	1.24(100)	1.95 (157)	0.138(11.11)
Saxidomus Nuttalli	—	—	0.22
Ostrea circumpicta	1.19(100)	2.106(176)	0.042(3.55)

From the above we find that the values of sodium and chlorine are rather close, but calcium in the oyster is only one third that given by the two other species. Regarding the high variability in calcium there is an interesting observation of COLLIP[1] on the coelomic fluid in *Mya arenaria* and *Cardium corbis*. COLLIP found that when these molluscs are exposed to the atmosphere or when they are kept in distilled water the amount of calcium shows considerable increase. Although magnesium also shows an increase under similar treatment, it is very slight when compared with that of calcium. I have shown some typical examples given by COLLIP in the following table.

Species	Ca per 100 cc.	Mg per 100 cc.	Remarks
Mya arenaria	mg. 35.4	mg. 49.0	Fresh. Kept some days in floating carriage off landing stage.
	92.0	79.0	24 hours in air at temperature of sea water.
	186.0	82.0	48 „ „
	220.0	—	72 „ „
	240.0	90.0	76 „ „
Cardium corbis	48.0	100.8	Fresh from sand beach.
	98.0	100.6	Kept 20 hours in air in laboratory.

As was mentioned, magnesium increases only very slightly but the calcium increases considerably, as the result of exposing the animals to the air. Whether or not the high calcium values obtained by MYERS can be interpreted from the results of COLLIP's investigation is difficult to decide.

QUINTON[12] noted that when *Ostrea edulis* was kept in diluted sea water (NaCl content$=2.37$ g.) for three hours NaCl concentration in the blood shows corresponding decrease.

The differences found by various investigators in the composition of various body fluids may either be due to the difference of species employed or to the different analytical method employed. For a definite statement, however, we must await future investigation.

BIBLIOGRAPHY.

1) BÁLINT, M., 1924. Eine jodmetrische Mikrobestimmung des Natriums. Biochem. Zeitsch., Bd. 150, S. 424–443.

2) KRAMER, B. and TISDALL, F. F., 1921. The direct quantitative determination of sodium, potassium, calcium, and magnesium in small amounts of blood. Jour. Biol. Chem., Vol. 48, pp. 223–232.

3) HAMMETT, F. S. and ADAMS, E. T., 1922. A colorimetric method for the determination of small amounts of magnesium. Jour. Biol. Chem., Vol. 52, pp. 211–215.

4) RUSZNYÁK, S., 1921. Eine Methode zur Bestimmung der Chloride in kleinen Flüssigkeitsmengen. Biochem. Zeitsch., Bd. 114, S. 23–26.

5) DENIS, W., 1921. Sulfates in blood. Jour. Biol. Chem., Vol. 49, pp. 311–317.

6) CLARK, E. P. and COLLIP, J. B., 1925. A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. Jour. Biol. Chem., Vol. 63, pp. 461–464.

7) OKAZAKI, K. and KOIZUMI, T., 1926. Über die Leibeshöhlenflüssigkeit von Holothurien, *Caudina chilensis* (J. MÜLLER). Sci. Rep., Tôhoku Imp. Univ., 4th Series, Biology, Vol. 1, pp. 139–142.

8*) GRIFFITHS, A. B., 1891. On the blood of the invertebrata. Proc. Roy. Soc. Edinburgh, Vol. 18, p. 288.

9*) GRIFFITHS, A. B., 1892. On the blood of the invertebrata. Proc. Roy. Soc. Edinburgh, Vol. 19, p. 130.

10) MYERS, R. G., 1919. A chemical study of the blood of several invertebrate animals. Jour. Biol. Chem., Vol. 41, pp. 119–135.

11) COLLIP, J. B., 1920. Studies on molluscan celomic fluid. Jour. Biol. Chem., Vol. 45, pp. 23–49.

12) QUINTON, R., 1900. Perméabilité 'de la paroi extérieure de l'invertébré marin non seulement à l'eau, mais encore aux sels. C. R. S., T. 131, p. 952.

* cited indirectly.

On Some Physico-chemical Properties of the Pericardial Fluid and of the Blood of the Japanese Oyster, *Ostrea circumpicta* PILS., with Reference to the Change of milieu extérieur.*

BY

MASAYASU YAZAKI.

(The Biological Institute, the Tôhoku Imperial University, Sendai, Japan).

Up to the present time several observers have examined some physico-chemical properties of the blood in Mollusca and in other invertebrates, but with regard to the pericardial fluid apparently no work has been published, as for as I am aware.

For this reason just stated, I have undertaken, at the suggestion of Prof. Dr. S. HATAI and Assist. Prof. K. OKAZAKI, the present work on the physico-chemical properties of the pericardial fluid and of the blood of the Japanese oyster, *Ostrea circumpicta* PILS.

METHODS.

To obtain the pericardial fluid the pericardium was carefully exposed and then its membrane was pierced with a pin, leaving the heart uninjured, and then the fluid was removed by means of a glass pipette.

The blood was collected directly from the heart also with a glass pipette after the pericardial fluid was taken. The fluids thus obtained were used for the purpose of the physico-chemical analysis in as fresh condition as possible.

Although in the case of the blood a single specimen was sufficient for single measurement, five or more specimens were usually necessary for the pericardial fluid.

The adult oysters used in this study were in a healthy condition. The specific gravity was determined by means of the OSTWALD

modification of the SPRENGEL pyknometer.

The specific conductivity was determined by the WHEATSTONE bridge method.

The freezing point depression was determined by the BECKMANN thermometer.

The surface tension was determined by the TRAUBE's Stalagmometer.

The viscosity was determined by the DETERMANN's viscosimeter.

The amount of NaCl contained in the fluids was determined by the RUSZNYÁK's method (1921).

COMPARISON BETWEEN THE GENERAL PROPERTIES OF THE PERICARDIAL FLUID AND OF THE BLOOD.

The amount of fluid contained in the pericardium of the normal individual fluctuates widely, ranging from 0.3 to 0.7 cc., but in the majority of cases it lies between 0.4 and 0.5 cc.. I have never found in my experience the refilling of the pericardium after the fluid has once been removed, even when hours and indeed in some instances 3 days elapsed. The blood obtainable from a single oyster varies from 4 to 7 cc..

The pericardial fluid is perfectly transparent and free from corpuscles, while the blood is somewhat opalescent, and the plasm, which was obtained after removing the corpuscles, exhibits the same colour as the blood. Sometimes, the blood is tinted with a faint yellowish green colour, but appears somewhat yellowish by transmitted light, and bluish by reflected light, just as DREW (1910) found on the blood of *Cardium*. On the other hand the pericardial fluid shows no alteration in its coloration even when the blood colour is yellow.

The reaction of both the pericardial fluid and the blood is neutral to litmus. The blood gives the strong protein reactions, such as the Biuret reactions and strong MILLON's reactions, while the pericardial fluid shows only the faintest reaction. DREW (1910) studied some proteid reactions and other properties of the blood of Lamellibranch, but no researches were made on the pericardial fluid.

On heating the blood gradually up to 72°.8-73°.7C., the cloudiness gradually deepens and then the precipitates are also formed gradually. The precipitates thus formed are identified as a protein, from positive

reactions shown by the various protein tests. This protein just mentioned is soluble in both acids and alkalies, and is reprecipitated on neutralisation. On the other hand, the pericardial fluid exhibits a faintly cloudy appearance on heating to 72°.3–73°.6C. Cuénot (1891) states in his paper on the blood proteins of several species of Mollusca that "un albuminoide....precipitable par l'alcool et les acides, coagulable par la chaleur à 74 degrés environ", but nothing was mentioned in regard to the pericardial fluid.

SPECIFIC GRAVITY.

Many investigators agree that the specific gravity of the blood of marine Invertebrates very closely resembles that of the sea water, showing that the former is in equilibrium with the latter, and, therefore, the concentration of the blood and other coelomic fluids will be altered by the change of the sea water until a new equilibrium is set up. It must be stated in this connection that comparatively few observations have been made on Molluscs.

The blood specific gravity of a gastropod, *Aplysia limacina* is 1.029, while that of the sea water is 1.029–1.037 (Bottazzi, cited after Quagliariello 1925).

In Lamellibranchiata, Paladino (1909) showed that the blood specific gravity of *Cardita sulcata* is 1.030, but no report is given concerning the sea water in which it lives.

In the oyster, I have found that the specific gravity of the pericardial fluid ranged from 1.022 to 1.024 and the blood from 1.023 to 1.028, while the sea water where the oysters live gave from 1.022 to 1.024. In some specimens, however, the value of the specific gravity of the fluids was shown beyond the limits just given above.

The values of the specific gravity for both pericardial fluid and blood of the oysters collected during the spring season are given in Table 1.

If we compare these data with those given in Table 2, which were obtained during the hot summer season, it will be noted that the specific gravity of the body fluids tends to be slightly higher in the summer season than in the spring.

In Table 2 are given the data obtained from the oysters collected

TABLE 1.

(Spring specimens)

No. of oysters	No. of determinations	Specific gravity	
		Pericardial fluid	Blood
35	6	1.022	1.023-1.025
95	74	1.023	1.025-1.027
75	54	1.024	1.025-1.028
Average 1.023			1.026

late in August. The specific gravity of sea water was found to be 1.023–1.025.

TABLE 2.

(Summer specimens)

No. of oysters	No. of determinations	Specific gravity	
		Pericardial fluid	Blood
45	17	1.022	1.025-1.026
60	31	1.023	1.025-1.027
85	49	1.024	1.025-1.028
145	63	1.025	1.026-1.030
15	12	1.026	1.028-1.029
Average 1.024			1.028

From Tables 1 and 2, we find that higher specific gravity of the sea water is associated with correspondingly higher specific gravity of the pericardial fluid and of the blood, and specific gravity of the pericardial fluid is always lower than that of the blood. This difference between the pericardial fluid and the blood is about 0.003.

If the oysters are kept in highly diluted sea water in which the specific gravity is correspondingly decreased, we find marked alteration in the specific gravity of both pericardial fluid and blood.

FRÉDÉRICQ (1885) found that the specific gravity of marine Crustacean blood varies similarly with that of the sea water.

GARREY (1905) found that when some lower marine Invertebrates were kept in diluted sea water the bodies were swollen and the concentration of the blood became low.

DAKIN (1908) found that when some marine Invertebrates are

placed in fresh water the body weight increased, but he did not determine the amount of body fluid contained.

I have also studied the influence of the diluted sea water (specific gravity 1.010–1.012) on the pericardial fluid and on the blood of the oysters, and the results of observations are given in Table 3 and Fig. 1.

TABLE 3.

No. of oysters	No. of determinations	Days of immersion in dil. sea water	Specific gravity	
			Pericardial fluid	Blood
15	6	0	1.024	1.029
,,	9	1	1.024	1.028
,,	9	2	1.024	1.026
,,	9	3	1.023	1.024
20	11	4	1.021	1.020
,,	11	5	1.020	1.019
,,	11	6	1.019	1.018
,,	11	7	1.016	1.018
,,	9	8	1.014	1.017
,,	9	9	1.014	1.017
,,	9	10	1.013	1.016
,,	9	11	1.012	1.016
,,	9	12	1.011	1.015

If the oysters are kept for 4 or 5 days in ½ diluted sea water, the specific gravity of blood becomes much lower than that given by the pericardial fluid, due probably to quicker decreases of the former than the latter. However, several days later, we find on the contrary that the blood gives higher specific gravity when compared with that of the pericardial fluid. This phenomenon just stated was produced probably because the decrease in specific gravity of blood become progressively slower contrary to the continuous decrease of pericardial fluid at a more uniform rate.

Beyond 4 or 5 days in the diluted sea water, the specific gravity of the blood becomes somewhat stationary, probably due to an existence of some regulative mechanism, as proposed by Höber (1902) in some teleosts, the precise nature of which is not yet known in Invertebrates. The existence of this regulative power just mentioned is highly probable as will be seen from the following experiments.

When the oysters, which had been kept for 10 days in a diluted
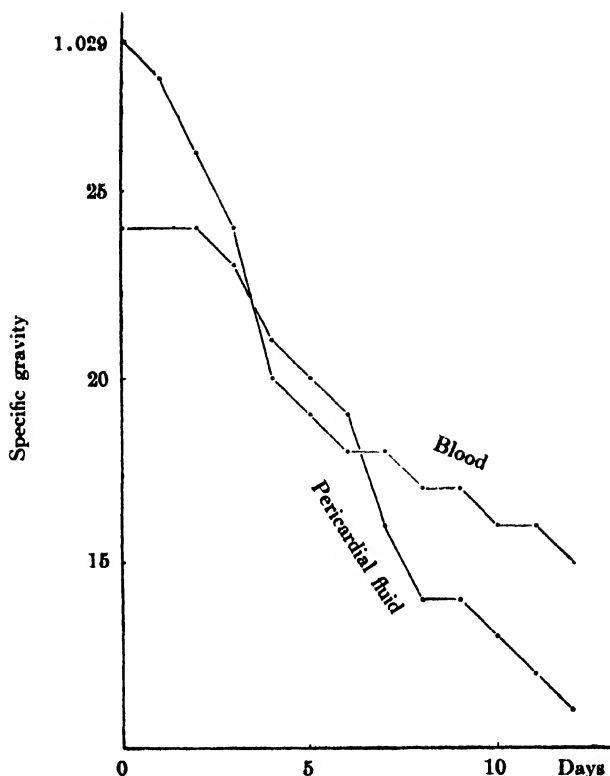
Fig. 1. Curve showing the change in the specific gravity of the pericardial fluid and of the blood of *Ostrea circumpicta*. The ordinates represent the specific gravity of the fluids, and the abscissae represent number of days the oyster was kept in ½ diluted sea water.

sea water, were returned into the normal sea water, a recovery of specific gravity of the pericardial fluid and of the blood also takes place very much more rapidly than the decrease in specific gravity.

Indeed, the specific gravity of the blood recovered its normality in from 10 to 13 hours or more, while in the pericardial fluid its recovery was several hours later than in the blood. The results of this experiment are given in Table 4.

This readjustment of the pericardial fluid and the blood to the normal sea water more rapidly than the adjustment to the diluted sea water seems to indicate the existence of some regulative mechanism

TABLE 4.

No. of oysters	No. of deter- mination	Times of immer- sion in normal sea water	Specific gravity	
			Pericardial fluid	Blood
10	4	0 hr.	1.012	1.015
15	9	1 ,,	1.012	1.016
,,	9	2 ,,	1.012	1.017
20	9	3 ,,	1.014	1.018
,,	9	4 ,,	1.015	1.020
15	9	5 ,,	1.015	1.023
,,	9	6 ,,	1.016	1.024
,,	9	7 ,,	1.017	1.024
,,	9	8 ,,	1.018	1.025
,,	9	9 ,,	1.019	1.026
,,	9	10 ,,	1.020	1.027
,,	9	11 ,,	1.022	1.027
,,	9	12 ,,	1.022	1.027
,,	9	13 ,,	1.023	1.028

in the body of the oyster. If such mechanism were wanting, the much slower decrease in specific gravity in the diluted sea water and such a rapid recovery in the normal sea water would be difficult to explain.

THE OSMOTIC PRESSURE OF THE PERICARDIAL FLUID AND OF THE BLOOD.

In 1871, BERT first found the osmotic relations existing between the body fluid and the surrounding water in several Invertebrates (Ecrevisse, Cyclopes, *Corethra plumicornis*, *Chironanus* etc.). Since that time the body fluids of a number of Invertebrates were tested in regard to the osmotic relation by numerous investigators. But, their observations were limited to the blood and thus no progress in this regard was made on the pericardial fluid.

In this matter, RODIER (1900) was probably the first to investigate the osmotic relations between the blood and pericardial fluid of the Selachians.

In Teleosts (*Cyclopterus lumpus*), DAKIN (1908) has shown that the pericardial fluid has its own equilibrium, the freezing point being a little lower than that of the blood.

BOTTAZZI (1897) studied the blood of several marine Invertebrates, and found that in many marine Invertebrates the freezing point of

the blood lies between -2.195 and -2.36°C. while that of the sea water lies similarly between -2.195 and -2.36°C., showing that the osmotic pressure of blood varies with that of the sea water. These observations of BOTTAZZI have also been proved by FRÉDÉRICQ (1904) to hold good in the case of Crustacea,

In 1904-1905, GARREY obtained very similar results from the studies of some Molluscan blood, and reported that "the blood or body fluid of a marine Invertebrate has the same freezing point as the sea water from which it is taken, no variation of more than two hundredths (0.02°C) of the degree being found".

DAKIN (1909) found that in the blood of *Pecten*, the freezing

TABLE 5.

No. of exp.	No. of oysters	No. of determinations	Pericardial fluid Δ	Blood Δ'	Sea water Δ''	Difference Δ'-Δ	Difference Δ''-Δ'
1	15	9	1.975	1.998	2.001	0.023	0.003
2	,,	9	69	63	1.990	-0.006	0.027
3	,,	9	7	84	1	0.017	0.007
4	,,	9	4	18	65	-0.046	0.047
5	,,	9	58	97	85	0.039	0.012
6	,,	9	38	4	2.005	0.056	0.011
7	,,	9	5	77	1.954	0.042	-0.023
8	,,	9	4	65	91	0.031	0.026
9	,,	9	28	5	85	0.035	0.020
10	,,	9	7	54	76	0.027	0.022
11	,,	9	5	6	81	0.031	0.025
12	,,	9	3	71	71	0.048	0.000
13	,,	9	18	67	83	0.049	0.016
14	,,	9	2	51	6	0.039	0.035
15	,,	9	08	43	5	0.035	0.042
16	,,	9	5	23	68	0.018	0.045
17	,,	9	4	37	4	0.033	0.027
18	,,	9	3	52	86	0.049	0.034
19	,,	9	1	22	67	0.021	0.045
20	,,	9	1.894	51	88	0.057	0.037
21	,,	9	0	22	2.008	0.032	0.086
22	,,	9	66	1.896	1.973	0.030	0.077
23	,,	9	2	920	82	0.058	0.062
24	,,	9	57	884	8	0.027	0.104
25	,,	9	4	900	75	0.046	0.075
26	,,	9	45	6	4	0.061	0.068
27	,,	9	2	1	1.877	0.059	-0.004
Range			1.842-1.975	1.884-1.998	1.897-2.008		
Average			1.913	1.947	1.979	0.034	0.032

point is sometimes higher and sometimes lower than that of the sea water.

In 1914, MONTI carried out a number of experiments on the osmotic pressure of blood of *Ostrea edulis* and *Mytilus*, etc. compared with the surrounding media, and found that the freezing point of the blood is much higher than that of surrounding sea water in *Ostrea* as well as in *Mytilus*.

In the Japanease oyster, on the contrary, the osmotic pressure of blood is generally higher than that of the pericardial fluid, and is usually lower than that of the sea water, indicating that both the pericardial fluid and the blood show a varying osmotic equilibrium with its surroundings, independently to each other, and therefore the two kinds of fluids are not in a state of osmotic equality with each other (Table 5).

It is evident from the table that the pericardial fluid and blood differ in their respective freezing point; −1.842 — −1.975 in pericardial fluid and −1.884 — −1.998 in blood, while the sea water of the aquarium in which the animals were kept freezes at −1.897 — −2.008. From these numerical relations we find that the freezing point of pericardial fluid is 0.034° higher than that of the blood, and the Δ of blood is 0.032° less than that of the sea water, though a reverse relation often occurs, as is shown by the oysters in experiments Nos. 2, 4, 7 and 27. Summarizing the above statements, the pericardial fluid of the oyster has its own equilibrium just as in the case of Teleosts, and the difference, though small, is present between the pericardial fluid and the blood, both of which are only separated by the thin membranes of the heart.

When the oysters were kept in $\frac{1}{2}$ diluted sea water, the blood osmotic pressure fell rapidly for about 5 days, and remained rather stationary up to 16th or 17th day, then was followed by another rapid fall (Fig. 2). This stationary period between about 5th and 17th days may be considered as indicating a maximal ability to regulate the osmotic pressure of the animal kept in a diluted sea water. This conclusion just given is based on a large number of data obtained from numerous other marine Invertebrates besides the oysters, which data will be given in another paper.

As was mentioned above, at the end of the stationary period a

TABLE 6.

No. of oysters	No. of determinations	Days of immersion in dil. sea water	Pericardial fluid Δ	Blood Δ
25	9	0	1.950	1.972
25	9	1 day	1.948	1.880
25	9	2 days	1.836	1.865
27	10	3 ,,	1.725	1.850
25	9	4 ,,	1.652	1.532
25	9	5 ,,	1.524	1.517
25	10	6 ,,	1.480	1.450
25	10	7 ,,	1.424	1.424
25	9	8 ,,	1.391	1.420
26	10	9 ,,	1.346	1.418
25	9	10 ,,	1.302	1.415
25	9	11 ,,	1.270	1.401
25	9	12 ,,	1.229	1.393
25	9	13 ,,	1.200	1.386
25	9	14 ,,	1.191	1.380
25	10	15 ,,	1.189	1.378
25	10	16 ,,	1.180	1.370
25	10	17 ,,	1.146	1.335
25	9	18 ,,	1.080	1.281
25	9	19 ,,	1.073	1.164
25	9	20 ,,	1.052	1.078
25	9	21 ,,	1.048	1.050·
25	9	22 ,,	1.044	1.041
25	9	23 ,,	1.040	1.034
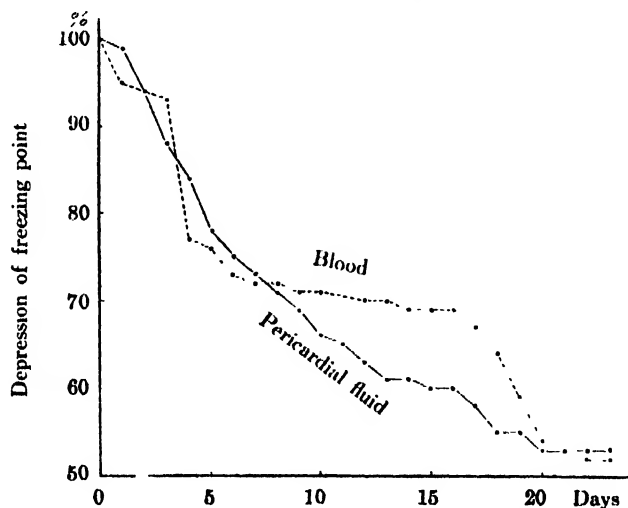
Fig. 2. The osmotic relation between the pericardial fluid and the blood of the oysters kept in diluted sea water (Δ=0.895). The immersion time in days are plotted as the abscissae, and the depression of freezing points (%) as the ordinates.

rapid decrease was shown.

On the other hand, the osmotic pressure of pericardial fluid shows gradual but steady decrease from the beginning to the end of the experiment, and such a stationary period as shown by the osmotic pressure of the blood is not shown owing probably to the less protective ability of the pericardium against the changed environments. The data of the experiments are given in Table 6 and Fig. 2.

THE QUANTITY OF NaCl CONTAINED IN THE PERICARDIAL FLUID AND IN THE BLOOD.

The quantity of NaCl contained in the blood of the oyster is intimately related to the external media. Some investigators claim that the chlorine value found in the blood of the oyster is higher than that of the sea water, while others found it less than that of the sea water. But concerning the pericardial fluid nothing was done so far as I am aware. Many investigators attempted to determine the relation of the salt contents of the blood to that of the sea water.

Cuénot (1891) has estimated the total salts in the blood of a number of marine Invertebrates in comparison with the sea water, and found that they were slightly less than those of the sea water in which the animals live.

Quinton (1900) found that the amount of NaCl contained in the blood of *Osterea edulis* is much less than that contained in the surrounding sea water, and, furthermore, that if the oysters are kept in diluted sea water, which contains 23.7 grms. of NaCl per 1000 cc., the salt contents of the blood becomes 23.1 grms. after 3 hours; and he extended similar studies to other Invertebrates, but did not determine the salt-content of other body fluids.

Frédéricq (1904) states that the blood and fluids contained in the body cavity, coelom, or haemocoel, of some marine invertebrates show almost the same percentage of salts as that of sea water, but nothing was mentioned about the oysters.

Drew (1910) states that the chlorine value of Lamellibranch blood is slightly higher than that of the sea water.

Monti (1914) states that the blood of *Ostrea edulis* has a higher concentration of salts than the sea water.

In order to compare accurately the quantity of NaCl contained in blood or pericardial fluid with that of the sea water, the animals under examination must be kept in the same conditions of sea water befor the samples are collected.   Keeping this point in mind, I carried out my experiment with the oysters.   That is, the oysters, which were kept for 24 hours in the normal sea water, which contained about 30.64–31.87 grms. of NaCl per liter, were transferred into slightly diluted sea water, which contained about 26.47–27.83 grms. of NaCl per liter, for 3 hours.   Under such circumstances it is shown that in the oysters, kept in the normal sea water, the amount of NaCl of the blood and of the pericardial fluid is less than that of the sea water, while in the oysters kept in slightly diluted sea water, it is greater than that of the sea water.   Following results will give the above relation in detail (Tables 7 and 8).

TABLE 7.

The oysters were kept in the normal sea water for 24 hrs.

No. of oysters	No. of deter- minations	NaCl in 1000 cc.		
		Pericardial fluid	Blood	Sea water
15	6	30.47 g.	31.12 g.	31.57 g.
15	6	29.59	30.51	30.42
15	6	29.34	29.89	30.64
15	6	29.25	30.62	31.08
15	6	28.48	29.41	30.86

TABLE 8.

The oysters were kept in the normal sea water for 24 hrs., then in diluted sea water for 3 hrs.

No. of oysters	No. of deter- minations	NaCl in 1000 cc.		
		Pericardial fluid	Blood	Sea water
15	6	30.76 g.	29.06 g.	26.63 g.
15	6	29.85	28.35	27.58
15	6	29.03	28.52	27.14
15	6	28.91	28.18	26.47
15	6	28.62	28.27	27.83

The above experiment shows that NaCl content in the body fluids can be altered by slightly diluting the sea water, and such degree of dilution may readily occur in the sea is not at all improbable. The different results obtained by the different investigators as to the NaCl content of the body fluids of various marine invertebrates might merely mean that the animals at the time of examination were living in sea water of various degrees of dilution.

The normal range of variation of NaCl in whole blood is from 27 to 31 grms. per liter, and in the pericardial fluid from 27 to 29 grms. per liter, while in the sea water from 29 to 31 grms. per liter.

PARTIAL OSMOTIC PRESSURE EXERTED BY SODIUM CHLORIDE.

The object of the following experiment is to determine whether there is any significantly greater variation in the freezing point depression according to individuals, when kept under the same physiological conditions, while the individual variation can be minimized; or further, whether the above relations are also shown in NaCl contained in the pericardial fluid and in the blood.

The analysis of the sea water of Mutsu bay, in which the oysters were collected, gave NaCl content to be about 29.00–30.57 grms. per liter and its freezing point was found to be from - 1.961° to 1.989°C. (mid point of range, 1.980°C.). The concentration of NaCl in terms of a gram-molecule will be 1.049 to 1.063; therefore from the above the osmotic pressure at the temperature of 0°C. should be about from 23 to 24 atmospheres.

In order to simplify the experiments, the writer, first of all, collected only those materials where the freezing point showed nearly identical values with each other and with that of the sea water, and a comparsion was made on the freezing point of the pericardial fluid with that of the blood in the same individual. Individual variation in the difference between the freezing points of the pericardial fluid and the blood fluctuated for a considerable range; in some samples the difference is 0.040°, while in the others it is 0.092. There are few cases the difference exceeds 0.100 degree. Most of the readings, however, varied between 0.05 and 0.06.

It will also be seen from the Tables 9 and 10 that the similar

relation as just mentioned exists in the variation of the calculated values of the part of the depression of freezing point caused by sodium chloride in the blood and the pericardial fluid.

The results are given in Tables 9 and 10. The value of Δ given in the tables is the mean of three readings of closer values, and the quantity of NaCl is the mean of at least three determinations.

TABLE 9.

No. of exp.	No. of oysters	No. of determinations	Pericardial fluid Δ	Amount of NaCl (grs. per liter)	NaCl Δ' Calculated	Δ'/Δ
1	15	6	1.943	28.63	1.804	0.92
2	15	6	1.942	28.07	1.768	0.91
3	15	6	1.927	28.23	1.778	0.92
4	10	3	1.920	28.75	1.811	0.94
5	10	3	1.912	28.48	1.794	0.93
6	10	3	1.908	28.16	1.774	0.92
7	10	3	1.907	28.62	1.803	0.94
8	10	3	1.903	28.55	1.799	0.94
9	10	3	1.889	27.58	1.738	0.92
10	10	3	1.885	28.41	1.790	0.95
11	10	3	1.884	27.81	1.752	0.92
12	10	3	1.883	27.16	1.711	0.90
13	10	3	1.868	27.04	1.704	0.91
14	10	3	1.852	28.41	1.790	0.97
15	10	3	1.851	28.56	1.799	0.97
16	10	3	1.850	28.00	1.764	0.95
17	10	3	1.846	27.27	1.718	0.93
18	10	3	1.840	28.34	1.785	0.97
19	15	3	1.814	27.49	1.732	0.95
20	10	3	1.810	27.77	1.750	0.91
Average	..	....	1.882	28.07	1.768	0.94

The blood of marine Invertebrates is generally considered to have a freezing point which is closely similar to that of the sea water, but so far as my experiments go, this statement does not seem to hold so strictly as is generally believed at least in the oyster.

In fact, there is a significant difference between the two, showing a difference of three hundredths (0.035°C.) of a degree in Δ corresponding to an osmotic pressure of about 0.42 atmospheres at 0°C. in favor of the sea water.

In the pericardial fluid, on the other hand, as will be seen from the above figures, the Δ is about six hundredths (0.063°C.) of a degree smaller than in the blood, and this would correspond to a difference

of osmotic pressure of approximately 0.75 atmospheres at 0°C. If the pericardial fluid is compared with the sea water, the difference of the two would be about 0.098 in Δ, which would correspond to 1.17 atmospheres at 0°C. in the osmotic pressure.

Furthermore a certain chemical difference is shown between the pericardial fluid and the blood; the amount of NaCl in the former ranges from 27.04 to 28.75 grms. per liter, while in the latter it is from 27.76 to 30.50 grms. Since the maximum content of NaCl in the pericardial fluid is 28.75 grams., and in the blood it is 30.50 grams, we would obtain, between the two, the difference of 0.111°C. in Δ and about 1.33 atmospheres at 0°C. in the osmotic pressure.

Thus the quantity of NaCl contained in the pericardial fluid and in the blood is different. In the tables, Δ represents the depression of freezing point of the blood or the pericardial fluid, and Δ' part of the depression to be caused by sodium chloride calculated from the amounts of the latter in these fluids. For the pericardial fluid we find the ratio $\Delta'/\Delta=0.94$, while for the blood we find the ratio $\Delta'/\Delta=0.93$.

TABLE 10.

No. of exp.	Blood Δ	Amount of NaCl (grs. per liter)	NaCl Δ' calculated	Δ'/Δ
1	1.983	30.05	1.893	0.95
2	1.984	28.74	1.810	0.91
3	1.966	29.72	1.872	0.95
4	1.970	29.66	1.868	0.94
5	1.946	29.24	1.842	0.95
6	1.971	29.81	1.878	0.95
7	1.954	29.96	1.887	0.96
8	1.972	30.50	1.922	0.97
9	1.951	28.40	1.789	0.92
10	1.953	29.37	1.850	0.94
11	1.942	28.46	1.793	0.92
12	1.937	27.91	1.758	0.90
13	1.960	29.42	1.853	0.95
14	1.924	27.76	1.749	0.90
15	1.917	29.62	1.866	0.97
16	1.918	29.70	1.871	0.97
17	1.916	28.91	1.821	0.95
18	1.902	29.57	1.863	0.98
19	1.905	28.48	1.794	0.94
20	1.926	28.86	1.818	0.94
Average	1.945	28.71	1.809	0.93

From the above, it may be said that 94% of the osmotic pressure of the pericardial fluid is exerted by sodium chloride, and hence the latter makes up 94% of osmotic concentration of the substances in the fluid. Similarly, the sodium chloride exerted 93% of the total osmotic pressure and makes up 93% of the osmotic concentration of the substances in the blood.

DUVAL (1928) reported that the NaCl in blood of *Buccinum undatum* (marine Gastropoda) is responsible to 95 per 100 of the osmotic pressure of the blood. His results in general accords with mine.

The results of my own researches show that the pericardial fluid of the oyster is not exactly isotonic with the blood, since its freezing point is higher than that of the blood (Tables 9 and 10).

INFLUENCE OF VARIOUS GRADES OF DILUTION OF SEA WATER ON ELECTRICAL CONDUCTIVITY.

BOTTAZZI (1905) studied the specific electrical conductivity and the freezing point of the blood in marine Invertebrates of several phyla, compared with those of sea water.

In the present investigation the writer has also determined the electrical conductivity of blood of several species with special reference to that of the oyster.

Determinations were made during the months of August and September. The animals after collection were kept in a large tub filled with sea water for one day before being used, in order to prevent the changes of salinity of sea water, though its gas content showed usually 4–6 per cent increase during the course of experiment, which however influences the result very slightly. The water temperature of the tub varied between 19°C. and 22°C.

I have attempted to determine whether or not the electrial conductivity of blood and that of sea water show any causal connection. As far as my observations went the existence of such relationship seemed highly probable, but for any definite statement a further accumulation of data is required. I have determined the electrical conductivity of blood and of the pericardial fluid of marine Invertebrates in comparison with sea water. The observations show that the electrical conductivity of the blood varies regularly with that of the sur-

rounding sea water as will be seen from Table 11 where a ratio k/k' (k = specific conductivity of blood, k' = specific conductivity of sea water) and a ratio Δ/Δ' (Δ = depression of freezing point of blood, Δ' = depression of freezing point of sea water) are given.

TABLE 11.

| Group | Species | Blood | | Sea water | | Δ/Δ' | k/k' |
		Δ	K(25°C.).10^6 *K(21°C).10^6	Δ'	K'(25°C.).10^6 *K'(21°C).10^6		
Coelen-terata	Anthopleura xanthogram-mica	1.967	48162	1.995	49513	.986	.973
	Cavernularia habereri	1.989	48924	1.995	49844	.997	.982
	Cribrina artemisia	1.941	48535	1.995	49513	.973	.980
	Dactylometra pacifica	1.970	47941	1.978	49575	.996	.967
Gephyr.	Sipunculus nudus*	2.265*	51200*	2.274*	54700*	.996	.936
Poly-chaeta	Aphrodite aculeata*	2.259*'	51700*	2.274*'	54700*	.993	.945
	Arenicola cristata	1.938	47898	1.986	49884	.976	.960
Echino-derma	Asterina pectinifera	1.974	48205	1.986	50637	.994	.952
	Caudina chilensis	1.884	47752	1.893	49924	.995	.956
	Heliocidaris crassispina	1.921	48464	1.950	49924	.985	.971
	Holothuria Poli*	2.299*	50300*	2.360*	55000	.974	.915
	Stichopus japonicus	1.980	47681	1.986	49879	.997	.956
Gastro-poda	Aplysia limacina*	2.317*	48100*	2.360*	55000*	.981	.875
	Aplysia sp.	1.923	47644	1.988	49896	.966	.953
	Dolium galea*	2.238*	50200*	2.360*	54700*	.984	.918
	Tugalia gigas	1.964	47897	1.987	49614	.988	.965
Lamelli-bran-chiata	Anadra inflata	1.947	47946	1.954	49865	.996	.961
	Modiolus capax	1.954	48129	1.961	49982	.996	.963
	Mytilus dunkeri	1.931	47513	1.982	49838	.974	.953
	Ostrea circumpicta	1.941	48134	1.988	49865	.976	.967
Cepha-lopoda	Octopus macropus*	2.290*	42200*	2.360*	55000*	.970	.767
	Octopus vulgaris*	2.296*	43800*	2.360*	55000*	.973	.796
Crusta.	Mitella mitella	1.989	47475	1.994	49807	.997	.953

* The data obtained by BOTTAZZI.

From the above table, it will be seen that among the animals studied, all show without exception a lower conductivity of blood than that of sea water, and the conductivity ratio k/k' shows no considerable variation among the different species, giving a small range of .875–

.982, with the exception of *Aplsia* and Cephalopoda which have much lower values and of those BOTTAZZI already gave much lower values. We find the range of Δ/Δ' to be .966–.997 without exception, and we further notice that when the ratio Δ/Δ' is compared with the conductivity ratio k/k', no definite relation is shown. However, in the oysters which were kept in the sea water of $\frac{1}{3}$ dilution the blood shows progressive decrease of conductivity in parallel to the depression of freezing point, and the same phenomenon is noted with that of the pericardial fluid. The data showing the relations found in the oysters are given in Table 12 and Fig. 3.

TABLE 12.

Effect of diluted sea water upon blood and pericardial fluid.

No. of oysters	No. of determinations	Days of immersion in dil. sea water	Pericardial fluid Δ	Blood Δ	Conductivity k(25°C.)	
					Pericardial fluid	Blood
15	9	0	1.948	1.992	48501×10^{-6}	49013×10^{-6}
20	18	1 day	1.908	1.880	$48203 \times$,,	$47815 \times$,,
10	20	2 days	1.850	1.600	$41551 \times$,,	$38649 \times$,,
15	21	3 ,,	1.681	1.517	$38462 \times$,,	$36944 \times$,,
15	18	4 ,,	1.602	1.501	—	—
20	21	5 ,,	1.500	1.470	$36224 \times$,,	$34745 \times$,,
20	21	6 ,,	1.384	1.450	$35646 \times$,,	$33211 \times$,,
25	15	7 ,,	1.324	1.420	$34230 \times$,,	$32600 \times$,,
20	13	8 ,,	1.227	1.402	$31736 \times$,,	$32408 \times$,,
15	10	9 ,,	1.212	1.384	$29875 \times$,,	$32129 \times$,,
20	10	10 ,,	1.150	1.349	$29108 \times$,,	$32011 \times$,,
20	10	11 ,,	1.111	1.337	$28640 \times$,,	$31876 \times$,,
20	10	12 ,,	1.089	1.325	$27362 \times$,,	$31422 \times$,,
15	10	13 ,,	1.063	1.317	$27325 \times$,,	$31057 \times$,,
15	10	14 ,,	1.051	1.310	$27281 \times$,,	$30824 \times$,,
15	10	15 ,,	1.030	1.304	$26800 \times$,,	$30413 \times$,,
15	10	16 ,,	1.025	1.300	$26220 \times$,,	$30246 \times$,,
15	10	17 ,,	0.986	1.280	$25834 \times$,,	$30014 \times$,,
15	10	18 ,,	0.952	1.199	$25301 \times$,,	$28727 \times$,,
15	10	19 ,,	0.924	1.107	$24773 \times$,,	$26252 \times$,,
15	10	20 ,,	0.904	1.078	$24145 \times$,,	$25038 \times$,,
15	10	21 ,,	0.883	0.923	$23766 \times$,,	$22111 \times$,,

As already mentioned, the oyster is one of the poikilosmotic animals, hence the concentrations of the body fluids vary correspondingly with variously diluted sea water; consequently the decreasing of the conductivity is proportional to the dilution of sea water. As is shown in Fig. 3, when the oysters were kept in $\frac{1}{2}$ diluted sea water for 5 or 6 days the conductivity of both fluids reduced about to one third,
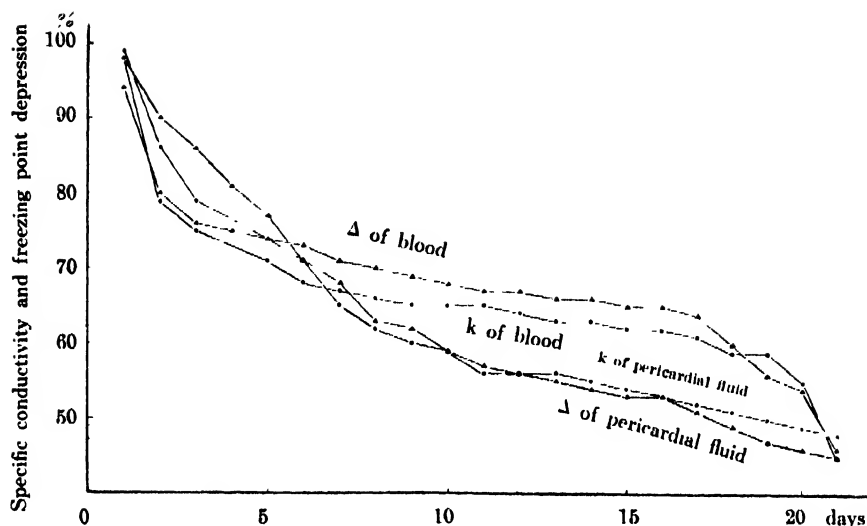
Fig. 3. Curves of the conductivity and the freezing point depression of the pericardial fluid and the blood of the oysters in the diluted sea water ($\Delta = 0.852$, $k_{25^\circ} = 23262 \cdot 10^{-6}$). The immersion days are plotted as the abscissae, and the conductivity and the freezing point (%) as the ordinates.

and after this period the conductivity of the blood decreases slowly while that of the pericardial fluid decreases somewhat faster; consequently a great disparity in conductivity occurs between the two as the time advances. Since there exists a parallelism between the decreasing of the conductivity and the depression of the freezing point in both the pericardial fluid and in the blood, we can predict the change of the blood if the change of pericardial fluid is known and vice versa.

THE RELATION BETWEEN VISCOSITY AND CONDUCTIVITY OF BLOOD OF SOME MARINE INVERTEBRATES, WITH SPECIAL REFERENCE TO THOSE OF PERICARDIAL FLUID AND BLOOD OF THE OYSTER.

The discovery by WIEDEMANN in 1856 of the fact that viscosity and conductivity are closely related, has aroused the deep interest of many investigators, and as a consequence a number of important results have been obtained since that time. In 1926 KUNITZ pointed

out the existence of a closer relation between viscosity and osmotic pressure, and showed that the values for the amount of solute in solution obtained from viscosity measurements may be calculated from the osmotic pressure measurements.   Moreover many valuable investigations on the viscosity have been performed by several authors, but the body fluids of marine Invertebrates still remain much to be studied.

In 1909, BOTTAZZI determined both the viscosity and the conductivity of blood of some marine Molluscs, but nothing was mentioned on the relation between the two.

QUAGLIARIELLO (1925) reexamined BOTTAZZI's data just mentioned, but likewise the relation existing between the two factors was untouched.

I found that the product of viscosity and conductivity or v. k is nearly constant in the majority of cases examined (see Table 13) though its true physiological significance is not yet clear.

The present writer undertook this observation with the hope not only to repeat some of the work of BOTTAZZI but to throw some light on the physiological meaning of v. k.   The value of v. k obtained from various groups of Invertebrate is given in Table 13.

TABLE   13.

Group	Species	$k_{25^\circ C}$	$v_{10 C}$	v. k
Coelenterata	*Anthopleura xanthogrammica*	48162.10^{-6}	1.274	0.061
	Cavernularia habereri	48924. ,,	1.240	0.061
	Dactylometra pacifica	47941. ,,	1.266	0.061
Polychaeta	*Arenicola cristata*	47898. ,,	1.375	0.066
Echinoderma	*Asterina pectinifera*	48205. ,,	1.320	0.064
	Caudina chilensis	47752. ,,	1.422	0.068
	Heliocidaris crassispina	48464. ,,	1.280	0.062
	Stichopus japonicus	47681. ,,	1.446	0.069
Gastropoda	*Aplysia* sp.	47544. ,,	1.361	0.065
	Tugalia gigas	47897. ,,	1.355	0.065
Lamellibranchiata	*Anadra inflata*	47946. ,,	1.371	0.066
	Mytilus dunkeri	47513. ,,	1.323	0.063
	Ostrea circumpicta	48134. ,,	1.302	0.063
	Pecten yessoensis	48572. ,,	1.422	0.069
Crustacea	*Mitella mitella*	47475. ,,	1.261	0.060

As will be seen from Table 13 the values of v. k. obtained from the blood of several marine Invertebrates lie between the two narrow limits 0.060 and 0.069.

In all the oysters examined the value of v. k or the product of conductivity and viscosity is slightly lower in the pericardial fluid than in the blood as will be seen from the following Table 14.

TABLE 14.

No. of oysters	No. of determination	Pericardial fluid			Blood		
		$k_{25°C}$	$v_{19°C}$	v. k	$k_{25°C}$	$v_{19°C}$	v. k
15	6	47563.10⁻⁶	1.273	0.062	48538.10⁻⁶	1.285	0.062
15	6	47565. „	1.300	0.062	49073. „	1.326	0.063
15	6	47820. „	1.280	0.061	48134. „	1.302	0.063
20	6	48176. „	1.255	0.060	49624. „	1.280	0.062

The values of v. k of the pericardial fluid of the oysters, similarly to the blood, come very close to the values given in Table 13.

This near constancy in the value of v. k suggests that the blood of the lower marine Invertebrates is essentially the same as to its properties as well as the concentration of electolytes contained.

The body fluids are sometimes less viscous, and sometimes more viscous than the sea water in which the animals live.

When the oysters were kept for 48 hours in sea water (relative viscosity 1.797) the blood became more viscous than the pericardial fluid (Table 15).

TABLE 15.

No. of oysters	No. of determinations	Subject	Relative viscosity (19°C)
	6	Sea water	1.797
40	6	Pericardial fluid	1.674
	6	Blood	1.719

When the oysters were transferred from the above sea water (v = 1.797) and kept for 24 hours in other sea water having the rela-

tive viscosity of 1.225 the blood became less viscous than the pericardial fluid (Table 16).

TABLE   16.

No. of oysters	No. of deter-minations	Subject	Relative viscosity (19°C)
	6	Sea water	1.225
40	6	Pericardial fluid	1.494
	6	Blood	1.472

On the contrary, after 48 hours in the above sea water (v = 1.225) the blood became more viscous than the pericardial fluid, but after this change the relative viscosity of the blood remained almost stationary as long as my observation lasted (about 4 days) (Table 17).

TABLE   17.

No. of oysters	No. of deter-minations	Subject	Relative viscosity (19°C)
	6	Sea water	1.225
40	6	Pericardial fluid	1.283
	6	Blood	1.302

During last summer the relative viscosity of the sea water kept in a tub varied from 1.204 to 1.685, but occasionally it surpassed the limiting values just given.

From the fact that change in the sea water produces corresponding change in the blood and pericardial fluid in regard to the viscosity and conductivity, it seems justified to conclude that there are some causal connections between these two factors just mentioned and composition of these body fluids.

ON THE SURFACE TENSION OF THE BODY FLUIDS OF MARINE INVERTEBRATES, ESPECIALLY THAT OF THE PERICARDIAL FLUID AND THE BLOOD OF THE OYSTER.

In the experiments already described it was shown that among inorganic salts contained in blood, pericardial fluid, and sea water, the

sodium chloride represents the greatest part and consequently it must play an important role on the surface tension.

It is a well-known fact that many inorganic salts or capillary-inactive substances raise the surface tension. The relative contents of these salts in body fluids and sea water are not far different from one another. If the salts are intimately related with the surface tension, then we may expect that the measure of the surface tension of those fluids closely resemble one another.

BOTTAZZI (1909) measured with great accuracy the relative surface tension for blood of some marine Invertebrates as well as of sea water.

The present writer also measured the surface tension with a series of some marine Invertebrates, especially with the oyster.

I shall first present the data on the oyster, as well as the method employed, in the following pages.

As a first step in the determination of the surface tension it was necessary to select the oysters in which the blood showed nearly equal electrical conductivity. The pericardial fluid was collected from those selected oysters. By this method, even a very slight difference in surface tension, if present, between the pericardial fluid and the blood can be determined quite clearly. The results of the investigation are given in Table 18.

TABLE 18.

No. of oysters	No. of determinations	Pericardial fluid		Blood		Sea water	
		Sp. conductivity (25°C)	Surface tension (19°C)	Sp. conductivity (25°C)	Surface tension (19°C)	Sp. conductivity (25°C)	Surface tension (19°C)
15	6	48257×10^{-6}	0.884	48874×10^{-6}	0.871	49250×10^{-6}	0.986
15	6	$48244 \times$ „	0.878	$48622 \times$ „	0.864	„	„
15	6	$47202 \times$ „	0.884	$48475 \times$ „	0.871	„	„
15	6	$48002 \times$ „	0.879	$48388 \times$ „	0.866	„	„
16	6	$47943 \times$ „	0.875	$48056 \times$ „	0.863	„	„

The following conclusions may be drawn from the results given in Table 18.

The blood exhibits a lower surface tension than that of the peri-

cardial fluid, while the sea water exhibits a higher surface tension than any of the others.

The changes of the specific conductivity and of the surface tension of the pericardial fluid do not run parallel with that of the blood, due probably to the difference of the protein content in both fluids. Since increase of the protein content lowers the surface tension, the difference in surface tension found between the pericardial fluid and the blood seems due to the difference of protein content rather than to the different amount of NaCl contained in those two fluids.

In the oysters which are kept in $\frac{1}{2}$ diluted sea water, a much clearer difference in the surface tension between the pericardial fluid and the blood is noted, as will be seen from Table 19.

TABLE   19.

No. of oysters	No. of deter-minations	Days of immer-sion in diluted sea water	Surface tension	
			Pericardial fluid (19°C)	Blood (19°C)
15	12	0	0.878	0.870
15	14	1 day	0.880	0.879
15	13	2 days	0.882	0.880
15	20	3 ,,	0.885	0.885
15	14	4 ,,	0.889	0.898
15	13	5 ,,	0.894	0.911
15	13	6 ,,	0.898	0.934
15	12	7 ,,	0.901	0.941
15	14	8 ,,	0.911	0.954
15	13	9 ,,	0.927	0.969
15	13	10 ,,	0.940	0.983

We find from Table 19 that the surface tension of the blood and of the pericardial fluid began to increase one day after the oyster was placed in the diluted sea water, and the rate of increase in the surface tension of the former is relatively quicker than that of the latter. In consequence of this difference, in 3 days its value became equal to that of the pericardial fluid, and then the difference in the surface tension between the blood and pericardial fluid become greater and greater, and the surface tension of the blood became considerably higher than that of the pericardial fluid at the end of the experiment, as is shown in Fig. 4.

I have also determined the surface tension of the blood in various
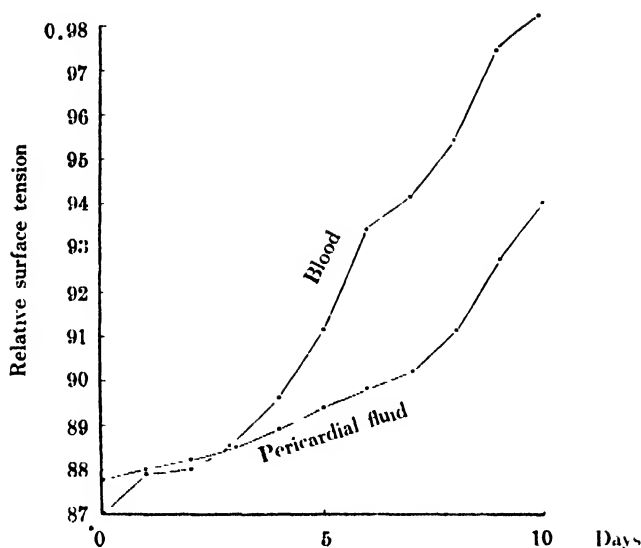
Fig. 4. Curves showing the change in the surface tension of the pericardial fluid and of the blood of *Ostrea circumpicta*. The ordinates represent the surface tension of the fluids, and the abscissae represent number of days the oyster was kept in ½ diluted sea water.

species of the marine Invertebrates together with that of the sea water where these are living and the results of determination are given in Table 20.

Despite the fact that the data on the surface tension given in Table 20 were obtained from the animals representing a wider range of taxonomical position, yet the values closely resemble one another and all show a definite difference from that of the sea water. Since the quantity of surface inactive substances contained in blood is about the same as in the sea water, and the surface tension is always lower in blood than in the sea water, it follows that the amount of the surface active substance must be much greater in blood, and again some slight individual variations found in regard to the surface tension depend much on the presence of a variable quantity of those substances.

It will be noticed from Table 20 that the relative surface tension of blood of many marine Invertebrates lies between 0.75 and 0.93, except Cephalopoda (0.68), giving the mean value of 0.8, while that of the sea water is 0.9.

TABLE 20.

Group	Species	Blood surface tension (19°C)	Sea water surface tension (19°C)
Coelenterata	*Cavernularia habereri*	0.933	0.947
	„ „	0.894	0.966
Polychaeta	*Arenicola cristata*	0.845	0.923
	„ „	0.908	0.937
Echinoderma	*Caudina chilensis*	0.822	0.898
	„ „	0.860	0.920
	Heliocidaris crassispina	0.897	0.949
	„ „	0.934	0.985
	Stichopus japonicus	0.889	0.946
	„ „	0.903	0.927
Gastropoda	*Aplysia depilans**	0.804*	0.982–1.003*
	„ *limacina**	0.754*	„
	„ sp.	0.822	0.988
	„ „	0.809	0.965
Lamellibran- chiata	*Anadra inflata*	0.783	0.918
	„ „	0.836	0.906
	Mytilus dunkeri	0.878	0.953
	„ „	0.825	0.922
	Ostrea circumpicta	0.854	0.972
	„ „	0.817	0.972
	Pecten yessoensis	0.849	0.937
	„ „	0.798	0.921
Cephalopoda	*Octopus macropus**	0.682*	0.982–1.003*

* The data obtained by BOTTAZZI.

CONCLUSION

From the present investigation the following results were obtained :

1.   The pericardial fluid of the oyster is perfectly transparent, while the blood is opalescent with a yellowish tint in transmitted light, and with a bluish tint in reflected light.

2.   The quantity of NaCl contained in the pericardial fluid of the oyster is less than that of the blood, and the latter is less than that of the sea water in which the animal lives.

3.   Amount of NaCl in the pericardial fluid of the oyster corres-

ponds to 94 per 100 of osmotic concentration of dry substances, while in the blood it corresponds to 93 per 100.

4. The specific gravity of the pericardial fluid of the oyster ranged from 1.022 to 1.024 (mid ponit 1.023) and that of the blood from 1.023 to 1.028 (mid point 1.026), while the sea water gave from 1.022 to 1.024 (mid point 1.023). These values are affected by the change in the surroundings when the animal is kept in $\frac{1}{2}$ diluted sea water.

5. The freezing point of the pericardial fluid of the oyster is about six hundredths (0.063°C.) of a degree higher than that of the blood; this difference corresponds to 0.75 atmospheres at 0°C.; consequently these fluids are not in a state of osmotic equality with each other.

6. When the oysters, which had been kept for a long period in a diluted sea water, were removed into the normal sea water, the specific gravity and the osmotic pressure of the blood and the pericardial fluid returned to their normal values more rapidly than they were changed by the external conditions when the animals were transferred from the normal to the diluted sea water. These changes were much slower in the pericardial fluid than in the blood. This phenomenon was due probably to the existence of some regulative power in the animal.

7. The product (v. k) of viscosity and conductivity of the blood is nearly constant in the majority of animals of different groups examined, and lies between the narrow limits 0.060 and 0.069. In the blood of the oyster the value of v. k was 0.063, while in the pericardial fluid it was 0.061.

8. The difference in the surface tension between the pericardial fluid and the blood seems due to the difference of protein-content rather than to the difference in amount of NaCl in those two fluids.

I wish to express my hearty thanks to Prof. Dr. S. HATAI and the late Assist. Prof. K. OKAZAKI, for their kind advice and review of the manuscript.

BIBLIOGRAPHY.

BAGLIONI, S. 1907. Einige Daten zur Kenntnis der quantitativen Zusammensetzung verschiedener Körperflüssigkeiten von Seetieren. Beit. Chem. Physiol. u. Path., Vol. 9, p. 50.

BARBOUR, H. G. and HAMILTON, W. 1924. Blood specific gravity: its significance and a new method for its determination. Am. J. Physiol., Vol. 69, p. 654.

BERT, P. 1871. Sur les phénomènes et les causes de la mort des animaux d'eau douce que l'on plonge dans l'eau de mer. C. R. S., Vol. 73, p. 382.

BOTTAZZI, F. 1897. Sur la pression osmotique de quslques sécrétions glandulaires d'invertébrés marins. Arch. ital. Biol., Vol. 28, p. 61.

BOTTAZZI, F. 1902. Contribution à la connaissance de la coagulation du sang de quelques animaux marins et des moyens pour l'empêcher. Arch. ital. Biol., Vol. 37, p. 49.

BOTTAZZI, F. 1905. Pressione osmotica e conduttività elettrica dei liquidi di animali acquatici. Arch. Fisiol., Vol. 3, p. 416

BOTTAZZI, F. 1906. Resistenza dei corpuscoli rossi di *Scyllium* et di *Sipunculus* a cedere rispettivament l'emoglobina e l'emeritrina. Arch. Fisiol., Vol. 3, p. 495.

BOTTAZZI, F. 1908. Osmotischer Druck und elektrische Leitfähigkeit der Flüssigkeiten der einzelligen, pflanzlichen und tierischen Origanismen. Ergeb. Physiol., Vol. 7, p. 246.

BOTTAZZI, F. 1909. Recerche sopra soluzioni di colloidi organici. Arch. Fisiol., Vol. 7, p. 579.

BOTTAZZI, F. 1923. On the surface tension of Protein solutions. Qur. J. exp. Physiol., Supl. Vol. p. 65.

COUVREUR, E 1902. Sur le sang des mollusques gastéropodes marins. C. R. Biol., Vol 54, p. 1251.

CUÉNOT, L. 1889. Études sur le sang, son rôle et sa formation dans la série animale. Deuxieme partie. Invertébrés. Arch. Zool. Exp, Vol. VII, p. 1.

CUÉNOT, L. 1891. Études sur le sang et les glandes lymphatiques dans la série animale. 2e partie: Invertébrés. Arch. Zool. Exp. Vol. IX, pp. 365, 593.

DAKIN, W. J. 1908. The osmotic concentration of the blood of fishes taken from sea water of naturally varying concentration. Biochem. J., Vol. 3, p. 258.

DAKIN, W. J. 1908. Variation in the osmotic concentration of the blood and coelomic fluids of aquatic animals, caused by changes in the external medium. Biochem. J., Vol. 3, p. 473.

DAKIN, W. J. 1909. L. M. B. C. Memoir, *Pecten*.

De BRUYNE, C. 1896. Contribution a l'étude de la phagocytose (1). Arch. Biol., Vol. XIV, p. 161.

DIMOOR, G. 1906. Rôle de la pression osmotique dans les phénomènes de la vie animale. Bull. Acad. roy. Belgique, No. 12. p. 857.

DREW, G. H. 1910. Some point in the physiology of Lamellibranch blood-corpuscles. Qur. J. Micro. Sc., Vol. 54, p. 605.

DUVAL, M. 1924. Réaction ionique du sang de quelques Invertébrés. C. R. S., Vol. 178, p. 1629.

DUVAL, M. 1924. Relation entre la concentration moleculaire du sang des Crustacés et celle du milieu extérieur. C. R. S., Vol. 178, p. 1754.

DUVAL, M. 1925. Recherches physicochimiques et physiologiques sur le milieu intérieur des animaux aquatique. Modifications sous l'influence du milieu extérieur. Ann. Intern. Océ., n. ser. p. 233.

DUVAL, M. 1927. Recherches sur le milieu intérieur de *Telphusa fluviatilis* LATR. Adaptation de ce Crustacé d'eau douce aux changements de salinité. Bull. Intern. Océ., No. 490, p. 1.

DUVAL, M. 1927. Sur la teneur en gaz carbonique total du sang des Invertébrés d'eau douce et des Invertébrés marins. C. R. S., Vol. 184. p. 1594.

DUVAL, M. 1928. Concentration moléculaire du sang de quelques mollusques d'eau douce. Ann. P. Physicochimie Biol. Vol. IV, p. 27.

FANO, G. and BOTTAZZI, F. 1896. Sur la pression osmotique du sérum du sang et de la lymphe en différents conditions de l'organisme. Arch. ital. Biol., Vol. 26, p. 45.

FRÉDÉRICQ, L. 1885, Influence du milieu ambiant sur la composition du sang des animaux aquatiques. Arch. Zool. Exp. Vol. III, p. XXXIV.

FRÉDÉRICQ, L. 1891. Sur la physiologie de la Branchie. Arch. Zool. Exp., Vol. IX, p. 177.

FRÉDÉRICQ, L. 1904. Sur la concentration moléculaire du sang et des tissus chez les animaux aquatiques. Arch. Biol., Vol. XX, p. 709.

FRÉDÉRICQ, L. 1904-'5 Note sur la concentration moléculaire des tissus solides de quelques animaux d'eau douce. Arch. Intern. Physiol., Vol. 2, p. 127.

FREUNDLICH, H. 1922. Colloid and Capillary Chemistry. Trans. by HATFIELD, S.

GARREY, W. E. 1904-'5. Osmotic pressure of sea water and of blood of marine animals. Including some observations on the permeability of animal membranes. Biol. Bull, Vol. VIII, p. 257.

GARREY, W. E. 1915. Resistance of Fresh-water Fish to changes in Osmotic and Chemical condition. Am. J. Physiol., p. 313.

GAUTRELET, J. 1903. Les pigments respiratoires et leurs rapports avec l'alcalinité apparente du milieu intérieur. Arch. Zool. Exp., Vol. 1, p. 31.

GRAM, H. C. and NORGAARD, A. 1923. Chloride and conductivity determinations on plasma. J. Biol. Chem., Vol. LVI, p. 429.

GROBBEN, C. 1886. Die Pericardialdrüse der Lamellibranchiaten und Gastropoden. Zool. Anz., Vol. IX, p. 369.

HENRI, V. and LABOU, S. 1903. Régulation osmotique du liquide interne chez les Oursins. Régulation osmotique des liquides internes chez les Holothuries. C. R. Biol., Vol. 55, p. 1224, 1244.

HÖBER, R 1902. Physikalische Chemie der Zelle u. der Gewebe.

KOVALEVSKY, A. 1889. Recherches sur les organes de l'excrétion.·Mollusques. Arch. Z. Exp., Vol. IX, p. XXXIX.

KUNITZ, M. 1926. An empirical formula for the relation between viscosity of solution and volume of solute. J. Gen. Physiol. Vol. 9, p. 715.

LOEB, J. 1918. The action of neutral salts on the osmotic pressure and other qualities of gelation. Proc. Soc. Exp. Biol., Vol. XV, p. 129.

MONTI, R. 1914. La variabilità della pressione osmotica nelle diverse specie animali. Atti. Soc. ital. Sc. nat., Vol. 53, p 301.

MYERS, R. G. 1920. A chemical study of the blood of several invertebrate animals. J. Biol. Chem., Vol. 41, p. 119.

NOLF, P. 1906-'7. Quelques observations concernant le sang des animaux marins. Arch. intern. Physiol., Vol. 4, p. 98.

OKAZAKI, K. and KOIZUMI, T. 1926. Über die Leibeshöhlenflüssigkeit von Holothurien, *Caudina chilensis.* Sci. Rep. Tohoku Imp. Univ. Biol. Vol. II, p. 139.

PALADINO, R. 1909. Vergleichung des Hämoglobins einiger Weichtiere mit dem der Wirbeltiere. Bioch. Zeits., Vol. 22, p. 495.

QUAGLIARIELLO, G. 1916. Recherche chimico-fisiche sui liquidi. Sulla reazione del sangue degli animali marini. St. Z. Nap. Vol. 1, p. 21.

QUAGLIARIELLO, G. 1925. Das Blut (Hamolymphe) der wirbellosen Tiere.-Mollusken. Wintersteins verg. Physiol., Vol. 1, p. 597.

QUINTON, R. 1899. L'invertébré marin fermé automatiquement au milieu extérieur lui est ouvert osmotiquement. Trav. Labor. Arcac., p. 27.

QUINTON, R. 1900. Communication osmotique, chez l'Invertébré marin normal, entre le milieu intérieur de l'animal et le milieu extérieur. C. R. S., Vol. 131, p. 905.

QUINTON, R. 1900. Perméabilité de la paroi extérieure de l'invertébré marin non seulement à l'eau, mais encore aux sels. C. R. S., Vol. 131, p. 952.

RODIER, M. E. 1899. Observation et expériences comparatives sur l'eau de mer, le sang et les liquides internes des animaux marins. Trav. Labor. Arcac., p. 103.

RODIER, M. E. 1900. Sur la pression osmotique du sang et des liquides internes chez les Poissons Sélaciens. C. R. S., Vol. 131, p. 1008.

RUSZNYÁK, S 1921. Eine Methode zur Bestimmung der Chloride in kleinen Flüssigkeitsmengen. Bioch. Zeits., Vol. 114, p. 23.

SHERRINGTON and COPEMAN, M. 1893. Variations experimentally produced in the specific gravity of the blood. J. Physiol., Vol. 14, p. 52.

STEWART, G. 1901. Die elektrische Leitfahigkeit tierischer Flüssigkeiten. Z. Physiol., Vol. 11, p. 397.

SUMNER, F. B. 1906. The osmotic relations between fishes and their surrounding medium. Biol. Bull., Vol. X, p. 298.

WITTING, G. 1860 Über das Blut einiger Crustaceen und Mollusken. Zeits. Wissensch. Zool., Vol. 10, p. 170.

The Normal and Abnormal Respiration in the Oyster, *Ostrea circumpicta* Pils.

By

Akira Nozawa.

(The Marine Biological Station of the Tôhoku Imperial University,
Asamushi, Aomori-Ken, Japan.)

Concerning the problems in relation to the respiration in the lower invertebrates much work has been done. Henze (1910) reports that the respiration rate of some of the simpler marine invertebrates (*Actinia, Anemonia, Sipunculus*) shows a dependence upon the oxygen tension in the water, decreasing as the latter decreases; whereas the respiration of higher invertebrates (*Carcinus Scyllarus, Eledone, Aplysia*) is independent of the oxygen tension. Krogh (1916) accepts Henze's interpretations and arrives with him at the general conclusion that the cold blooded animals are independent of the oxygen tension in the surrounding medium except in some of the simpler organisms. Bruce (1926) reports that the absorption of the oxygen and the output of the carbon dioxide differ with different temperatures, that is, higher temperature increases both the absorption and output, though this process cannot go on beyond a certain limit with further increase of temperature. Regnauld and Reiset (1849) report that the respiratory quotient of *Ostrea edulis* is 0.76 (15°C.). Parker (1922) measured the metabolic rate in four states, relaxed, contracted, relaxing, and contracting of the sea anemone, *Metridium marginatum*, and found that in the relaxed, contracted, and relaxing states the amounts of carbon dioxide excreted were found to be about the same, but in the contracting state showed an appreciably higher increase. Parnas (1910) reports that an *Anodonta*, the adductor muscles of which had a section area of 0.3 sq. cm., calls forth no increase in the respiratory exchange either during or after the loading with 3000 gms. for three hours.

The present investigation was carried on with a hope to obtain further data on the respiration of the oyster subjected to severe strain by hanging on it a heavy weight.

I wish to express my sincere thanks to Prof. S. HATAI and the late Assistant Prof. K. OKAZAKI for their valuable suggestions and criticims throughout the course of this study.

METHOD.

The determination of the oxygen and the carbon dioxide was made by the VAN SLYKE method.

The oyster was placed in 8 liters of sea water held in a jar with a volume of about 10 liters, and liquid paraffin was used to intercept the sea water from the air. It is often stated that air can permeate through liquid paraffin, and I tested this assumption just stated qualitatively by means of the methylene blue method. To 20 cc. of 1% methylene blue dissolved in sea water is added 1% rongalit solution until the colour disappears. This mixture is covered by the liquid paraffin and the amount of time which is necessary to recover the blue colour in the solution is determined. It was found from this test of the liquid paraffin, covering the medium to the thickness of 3 cm., that the interception between the atmosphere and the medium is complete for nearly 24 hours.

The outer layer of the oyster shell is usually infested with numerous organisms, including worms, and therefore these should be removed completely before the oyster is used, so as to prevent the respiration of these extraneous organisms, for otherwise the result would naturally be inaccurate. However, complete clearing of these adhering organisms is practically impossible, and therefore I covered the shells with soft paraffin, which, though simple, was found to be very effective.

A sample of the sea water was taken from the jar by means of a pippet one opening of which is joined to a U-shaped glass tube which is partially filled with mercury, and another end is connected to capillary tube which is inserted into the jar through the liquid paraffin, and the required amount of sea water is automatically sucked into this pipette through the capillary by properly lowering or raising the mercury.

RESULT OF THE EXPERIMENT.

1. NORMAL RESPIRATION.

Gas analysis of the water was made once for every 2 hours. As an example I have given the data obtained from one oyster in Table 1 and in Fig. 1. As is shown in Table 1 and Fig. 1, the oxygen is consumed nearly at the same rate during the first 8 hours of the experiment until the oxygen tension becomes about 0.1%. This relation indicates that during this first 8 hours the rate of the oxygen consumption is practically independent of the oxygen tension in the medium and is maintained at the same rate. It is to be noted that during this period just mentioned the rate of carbon dioxide production is also approximately uniform, similar to that shown by the oxygen consumption. But after 8 hours of the experiment the output of the carbon dioxide shows a greater rate of increase as compared with that of the consumption of oxygen. BERKELEY (1923) reports that the crystalline style in certain molluscs is related to the anaerobic respiration, from the fact that in a mollusc under anaerobic condition for eight days the crystalline style disappears entirely. I also noted in my experiment that it disappeared after 24 hours, and in some others

TABLE 1.

Time in hours	O_2 content Vol. %	O_2 consumed Vol. %	CO_2 content Vol. %	CO_2 evolved Vol. %	Respiratory quotient
0	0.739		3.985		
2	0.553	0.186	4.159	0.174	0.860
4	0.392	0.161	4.296	0.137	0.850
6	0.217	0.175	4.467	0.175	1.000
8	0.114	0.103	4.671	0.204	2.001
10	0.082	0.032	4.761	0.091	2.843
12	0.064	0.017	4.814	0.052	3.058
14	0.051	0.014	4.879	0.065	4.644
16	0.050	0.001	4.954	0.075	7.500
18	0.043	0.007	4.989	0.035	5.000
20	0.040	0.003	5.006	0.017	5.666
22	0.035	0.005	5.038	0.032	6.040

Total weight .... .286 gms.
Length .... 13 cm.
Width .... 8 cm.
Fresh body weight without shell .... 38 gms.
Temperature of sea water in the jar .. . . .22°C.

even in 12 hours. This disappearance of the crystalline style in my
test cannot be taken as positive proof that it was used up during
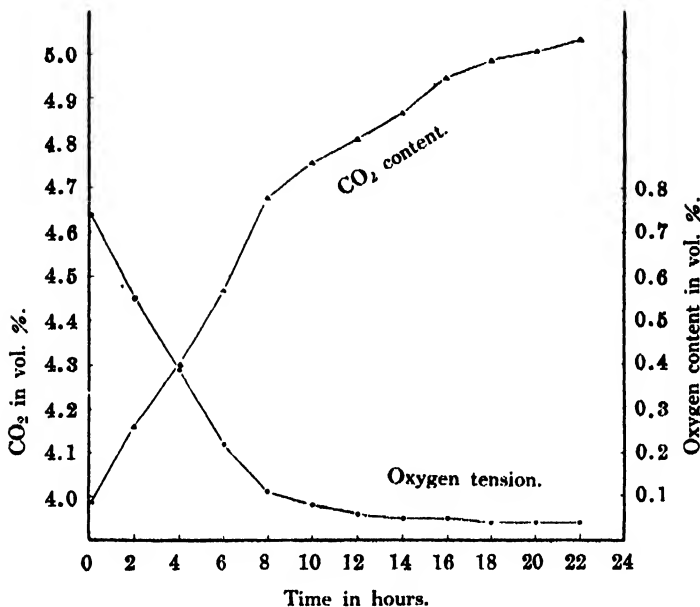
Fig. 1. Normal respiration.
CO₂ content and oxygen content in the medium.

anaerobic respiration, since its presence or absence in the oyster was
unable to be determined until the experiment was over. However,
exposing the oyster for 3 to 4 hours in the atmosphere in which a
presence of the crystalline style is known, brought about complete
disapperance in most of them. Therefore, its connection with anaerobic
respiration cannot be doubted. The rates of the consumption of oxygen
and of the output of the carbon dioxide decrease more and more
every hour, and on the other hand the respiratory quotient becomes
larger and larger every hour. Two hours after the experimentation
the respiratory quotient was found to be 0.873, while REGNAULD and
REISET (1849) found it to be 0.79 in *Ostrea edulis* and 0.84 in *Mytilus
edulis*. These differences in the values of respiratory quotient just
given may be due to the difference of species used, but needs further
observation before a definite statement can be made.

The respiration carried at different temperatures has been observed by BRUCE (1926). He reports that the respiratory activity of *Mytilus* is optimum at 22.3°C., but at higher than 25°C. or at lower than 21°C. is gradually decreased. GALTSOFF (1928) found with *Ostrea virginica* GM. that the optimum temperature for the mechanical activity of the gills lies between 25°C. and 30°C. Below 5°C. no current is produced,

TABLE 2.

Mean value of oxygen uptake per 10 gms. wet tissue per hour.	Water temperature in °C
0.052	10.0
0.078	15.2
0.102	22.0
0.135	25.0
0.115	27.0
0.080	30.0
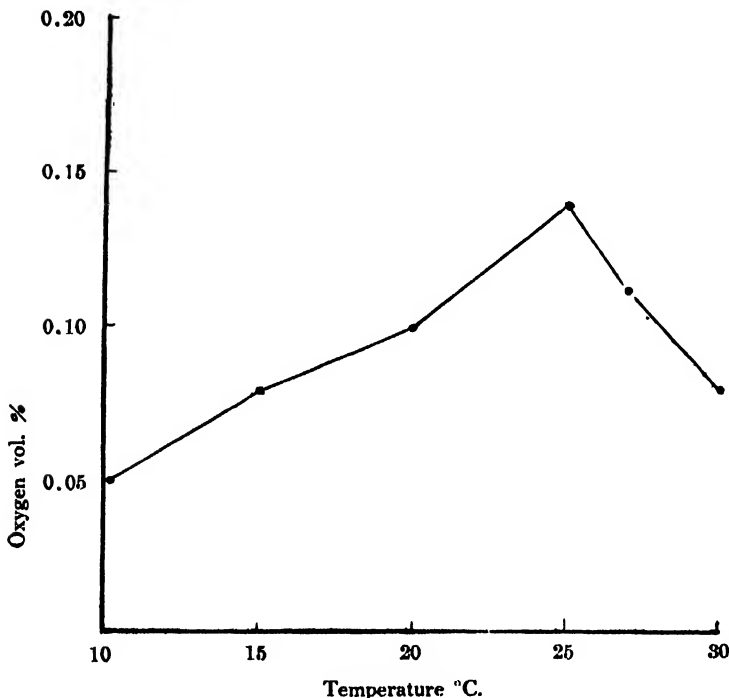
Fig. 2. Mean values of oxygen uptake per 10 gms. wet tissue per hour.

though the cilia are beating. Ciliary motion stops entirely at the freezing temperature of sea water. I have also found a similar relation, as will be seen from Table 2 and Fig. 2.

As is shown in Table 2 and in Fig. 2, *Ostrea circumpicta* Pils. showed the optimum respiratory activity at 25°C., which in turn at 30°C. fell suddenly. From 10°C. to 25°C. the activity increased gradually. The values given by the oyster are 2° to 3°C. higher than the optimum activity shown by *Mytilus*. This difference may be due to the adaptation to their respective surroundings, for the temperature of the sea water where *Mytilus* was collected is 14°C. at its highest, while the sea water of The Asamushi Marine Biological Station where the oyster is living is 23°C. at its highest. TAKATSUKI ('29) recently found that the heart pulsation stops at 5°C. and at 45°C. in the oyster of temperate zone (*Ostrea circumpicta* Pils.) and at 9°C. and at 50°C. in the oysters of tropical zone (*Ostrea dendata* Küster). This is another example of an adaptation of the oysters to their surroundings.

The value of pH in the medium. (sea water) where the oyster was kept was determined colorimetrically. The normal reaction of the sea water in Mutsu Bay ranges from pH 8.15 to pH 8.2. 3 liters of the
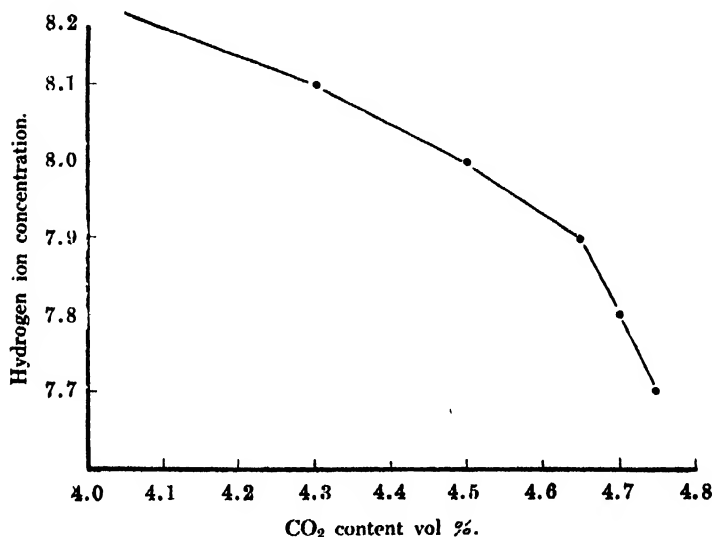
Fig. 3. The relationship between CO_2 content and the hydrogen ion concentration.

sea water in which a oyster was kept for 24 hours showed an increase of acidity to pH 7.7 (Fig. 3). This slight increase of acidity notwithstanding that the oyster excretes a great amount of carbon dioxide, is due to the production of carbonate, thus reducing the acidity of the medium.

The opening and closing movement of the oyster shell is rather irregular but when the oxygen content is normal, it is about six times per hour. If however the oxygen content reduces to 2 to 3 cc. per liter, the frequency of shell movement is much reduced, and the time required for opening and closing becomes correspondingly longer. As is shown in Fig. 4, if the oxygen content reduced to 1 or 1.5 cc. per liter, the movement of the shell stops permanently.
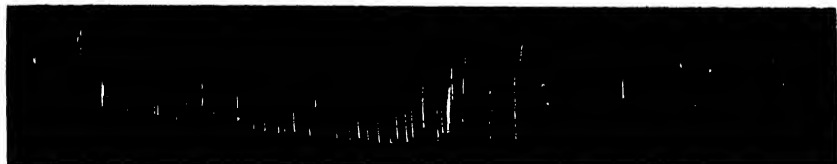
Fig. 4. The shell movement of the oyster in normal state.
Ascent of curve shows closure of shell.
Time 8 hours.

2. THE RESPIRATION UNDER ABNORMAL CONDITION.

To one side of the shell a heavy weight (5 kgs.) was hung, the other side was fixed horizontally in position. The oxygen consumption and the carbon dioxide output under such circumstances were compared

TABLE   3.

Time in hours	O_2 content vol %	O_2 consumed vol %	CO_2 content vol %	CO_2 evolved vol %	Respiratory quotient
0	0.658		3.929		
2	0.423	0.235	4.114	0.183	0.780
4	0.274	0.145	4.225	0.113	0.785
6	0.173	0.105	4.359	0.134	1.275
8	0.118	0.057	4.528	0.171	3.000
10	0.089	0.029	4.635	0.107	3.689

Total weight ....298 gms.
Wet tissue weight .... 31 gms.

with the normal respiration.   As is shown in Table 3 and Fig. 5, the rate of the oxygen consumption during the abnormal respiration is nearly the same as that found in the normal respiration, and consumes at the uniform rate until it reduces to 1.8 cc. per liter, but is about 1.3 or 1.2 times greater than that of the normal respiration.

The respiratory quotient during the first 4 hours is smaller than
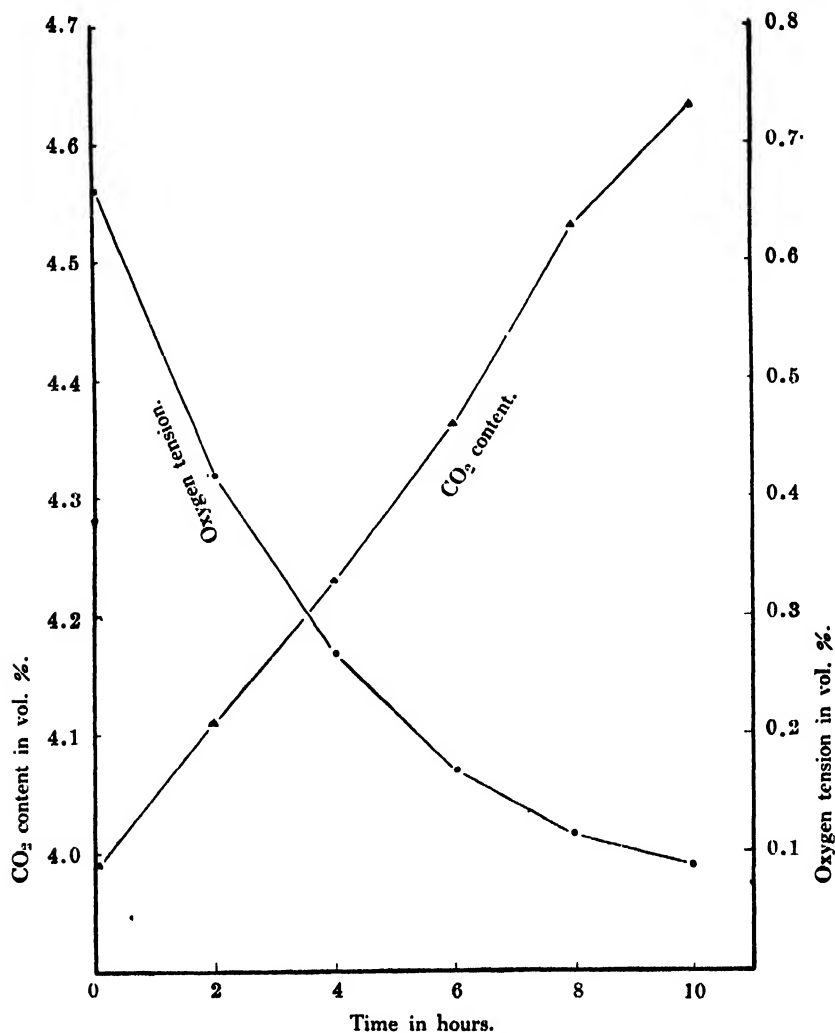
Fig. 5. CO_2 content and Oxygen content in the medium.

that of the normal respiration, owing probably to greater oxygen consumption necessitated physiologically by the heavy weight attached to the shell. After 3 or 4 hours the respiratory quotient increases rather suddenly, owing to a smaller oxygen consumption associated with a considerably greater amount of carbon dioxide output. This sudden increase of the carbon dioxide output just stated is probably an additional production of the carbon dioxide from the decompostion of the lactic acid. As was already stated the crystalline style seems somehow related to the anaerobic respiration, as my test shows its disappearance after exposing the oyster 3 to 4 hours in the atmosphere. Thus 18 hours after the experiment a disproportionally greater amount of the carbon dioxide eliminated for a negligibly smaller absorption of the oxygen might be explained that the crystalline style was utilized for this anaerobic respiration. BERKELEY (1923) reports that guaiacum, which is contained in the crystalline style, is capable of oxidizing the products of the metabolic activity of the tissues of the animals and thereby playing a part in anaerobic respiration. If BERKELEY's hypothesis is true, the greater amount of carbon dioxide excreted by the oyster under extreme conditions becomes highly probable (Table 1 and 2). It seems to me also probable that since a larger amount of carbon dioxide is normally found combined as carbonate in the blood and tissue fluid, CO_2 may in turn be produced from 'this source just mentioned if a sufficent amount of acid was produced as the result of heavy load.
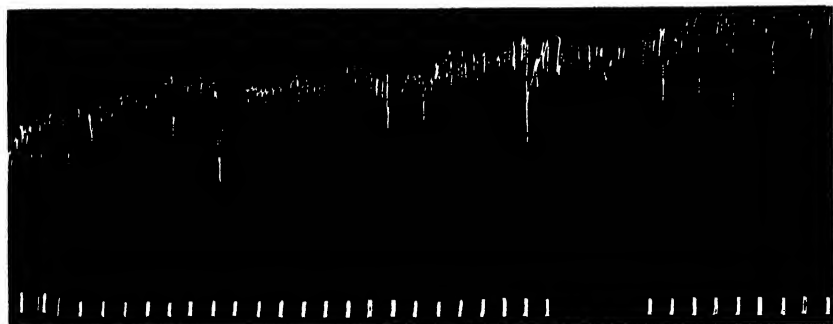
Fig. 6. The shell movement of the oyster in abnormal state.
Descent of curve shows closure of shell.
Time marked per 15 minutes.

The adductor muscle of the oyster to which a heavy weight of 5 kgs. was hung, ruptured in 7 or 8 hours in almost all cases. The movement of the shell differs from that of the oyster under normal condition, as is shown in Fig. 6. The oyster on which 5 kgs. are hung performs a complete movement of opening from 10 to 15 times per hour, mixed with a much wider opening of the shell occasionally. Even under such strain of heavy loading the shell open slowly and closes quickly as is the case with the normal oyster. After 5 to 6 hours, the oyster keeps the shells open partially and never closes entirely, till the adductor muscle at last is severed. PARNAS (1910) shows quite conclusively that the shortened state of the tonus muscle, produced for instance by a weight hung on the valves of a clam, calls for no greater expenditure of energy than does the unloaded state. PARKER (1922) obtained similar results, tested on the sea anemone, and concluded that the respiratory activity is the largest in the contracting state. In my experiment, the shell movement under loading is much accelerated compared with that unloaded. If we now accept PARKER's view that during contraction more carbon dioxide is produced than at contracted, relaxed, and relaxing states, we should anticipate in the present experiment of heavy loading a greater amount of carbon dioxide excretion, since under such a condition frequency of the shell movement becomes greater than unloading; in other words, the number of the times the muscle is contracting and relaxing itself is greater, thus producing greater amount of carbon dioxide correspondingly. In fact, we obtained a greater amount of carbon dioxide under an abnormal condition, such as shown in the present experiment, and support PARKER's observation on the sea anemone.

SUMMARY.

(1) The gaseous metabolism is accelerated under the abnormal condition compared with that under the normal condition.

(2) The respiratory quotient at the earlier stage of the oyster on which a heavy weight was hung is smaller than that of an oyster without load, but the former suddenly increases over the latter after some hours.

(3) The gaseous metabolism of the oyster differs at different

temperatures: the metabolism being diminished at both lower and higher temperatures than 26°C.

(4) The rate of the oxygen consumption in the oyster is independent of the oxygen tension till its pressure is reduced to 0.1% or below, and although normally in the oyster the carbon dioxide output is parallel to the oxygen consumption, even when the oxygen consumption is reduced to none, the carbon dioxide increases.

BIBLIOGRAPHY.

1) AMBERSON, W. R., MAYERSON, H. S. and SCOTT, W. J. 1924. The influence of oxygen tension upon metabolic rate in invertebrates. Journ. gen. Physiol., Vol. 7, No. 1, pp. 171–176.

2) BERKELEY, C. 1923. On the crystalline style as a possible factor in the anaerobic respiration of certain marine Mollusks. Journ. exper. Zoöl., Vol. 37, pp. 447–488.

3) BRUCE, J. R. 1926. The respiratory exchange of the mussel (*Mytilus edulis* L.). J. Biol. Chem., Vol. 61, pp. 522–573.

4) GALTSOFF, P. S. 1928. The effect of temperature on the mechanical activity of the gills of oyster (*Ostrea virginica* GM.). Journ. gen. Physiol., Vol. XI, No. 4, pp. 415–431.

5) HENZE, M. 1910. Über der Einfluss des Sauerstoffdrucks auf den Gaswechsel einiger Meerestiere. Bioch. Zeits., Bd. 26, S. 255–278.

6) KROGH, A. 1916. The respiratory exchange of animals and man. Monographs on biochemistry. London.

7) NOMURA, S. 1926. The influence of oxygen tension on the rate of oxygen consumption in *Caudina*. Sci. Rep., Tôhoku Imp. Univ., Fourth Series, Vol. 2, pp. 133–138.

8) PARKER, G. H. 1922. The excretion of carbon dioxide by relaxed and contracted sea Anemones. Journ. gen. Physiol., Vol. 5, No. 1, pp. 45–64.

9) PARNAS, J. 1910. Energetik glatter Muskeln. Pflüger's Arch., Bd. 134, S. 441–495.

10) REGNAULT et REISET. 1849. Recherches chimiques sur la respiration des animaux. Ann. de chim. et de Phys., T. 26.

11) TAKATSUKI, S. 1929. The heart pulsation of oysters in tropical seas compared with that of those living in seas of the temperate zone. Records of Oceanographic Works in Japan, Vol. 1, No. 3, pp. 102–112.

12) TORYU, Y. 1927. The respiratory exchange in *Carassius auratus* L. and gaseous exchange of the air bladder. Sci. Rep., Tôhoku Imp. Univ., Fourth Series, Vol. III, pp. 87–96.

13) VAN SLYKE, D. D. 1924. On the determination of gases in blood and other solutions by vacum extraction and manometric measurement. II. J. Biol. Chem., Vol. 61. pp. 575–584.

Studies on the Digestive Enzymes
of *Ostrea circumpicta.*

By

Eishirô Sawano.

(The Marine Biological Station of the Tôhoku Imperial University,
Asamushi, Aomori-Ken, Japan.)

Introduction

Many investigations have been made dealing with the mode of feeding and physiology of digestion in molluscs, including several species of Lamellibranchia. Fredericq (1878) in *Mya* and *Mytilus;* Mitra (1901) in *Anodonta*; Roaf (1908) in *Pecten;* Dakin (1909) in *Pecten;* Yonge in *Mya* (1923) and in *Ostrea* (1926). But in all these reports cited the characteristic properties of the enzymes concerned with the digestion have not always been given, and especially that of the proteolytic enzyme is unsatisfactory. Yonge has recently studied this in detail, but very little attention was given to the protease with the assumption that the oyster does not feed on animal matter.

The present work was undertaken as a part of the collaboratory works on the physiology of oyster carried in our Biological Institute, and has been carried on from July to October 1928, at the Marine Biological Station, Asamushi, Aomori-ken.

I wish to express here my gratitude to Prof. Hatai for his kindness in revising the manuscript, and also to the late Assist. Prof. K. Okazaki, and Assist. Prof. S. Kokubo at the Marine Biological Station for their helpful suggestions.

Experimental Data.

Distribution of Amylase in the Body
of *Ostrea circumpicta.*

Presence of amylase was tested with various portions of the oyster which were treated in the following manner:

(1) Blood as well as pericardial fluid was centrifuged before its use, so as to remove various corpuscles present in it, (2) fresh crystalline style was dissolved in distilled water to the concentration of 2 per cent., (3) gastric juice was diluted to 10 per cent with distilled water, (4) digestive diverticula, and the other tissues such as muscles, organs, etc. were separately ground in a mortar and extracted with distilled water of 3 times its weight and the extracts were used for the test. The results obtained are shown in Table 1.

TABLE   1.

	Cryst. style	Gastric juice	Digest. div.	Gast. wall	Adduct. muscle	Heart	Blood	Pericard. fluid
Achrom. point	30 m.	1 h.	30 m.	18 h.	3 h.	20 h.	30 h.	—
Reduct.	marked	marked	marked	weak	marked	weak	very weak	negative
Control	negative	trace	weak	trace	trace	trace	negative	negative

The mixture of 1 cc. sample + 1 cc. 1% starch solution + 2 cc. 1% NaCl was kept in a water bath at 35°C. Achromic point was determined by dropping from time to time a single drop of the mixture into 1 cc. of acidified 0.001 N iod solution, and the time required for the achromic point of the mixture was noted. The reducing action was tested after 24 hours of digestion by BENEDICT's solution. For the control boiled samples similarly treated were invariably set up. Toluol was used as the antiseptic.

MILK CLOTTING ACTIVITY IN THE PARTS OF *OSTREA CIRCUMPICTA*.

The milk clotting activity was tested by using calcified milk. The results obtained are shown in Table 2. Samples were prepared in the same manner as was described above.

The mixture of 1 cc. sample + 1 cc. calcified milk (50 cc. milk + 10 cc. N. $CaCl_2$ + 40 cc. distilled water) was warmed in a water bath at 35°C. At the given time the state of the mixture was observed.

TABLE 2.

	Cryst. style	Gast. juice	Digest. div.	Gast. wall	Muscle	Heart	Blood	Pericard. fluid
1 hour	clot	clot	clot	unchang-ed	unchang-ed	unchang-ed	unchang-ed	unchang-ed
2 hs.	not resolved	not res.	not res.	clot	unch	unch.	unch.	unch.
3 hs.	not resolved	not res.	partly res.	not res.	clot	clot	unch	unch.

INFLUENCE OF pH ON THE ACTION OF AMYLASES.

I. Optimum pH for the amylase in crystalline style.

The optimum pH for the amylase from crystalline style was determined. The method and results are shown in Table 3.

TABLE 3.

Flask	pH of the mixture	Glucose in per cent.
1	4.0	0.0138
2	4.8	0.0726
3	5.6	0.1820
4	6.0	0.2092
5	6.6	0.1985
6	7.0	0.1872
7	7.55	0.1764
8	8.1	0.1240
9	9.0	0.0632

To each of the mixtures of 10 cc. 2% crystalline style solution + 25 cc. 2% NaCl was added 10 cc. of buffer solution, giving different degrees of pH. Altogether 9 different mixtures were prepared, and were incubated for 6 hours at 37–38°C. The nine different buffer solutions were prepared after SÖRENSEN's method. The pH of the mixed solutions was determined colorimetrically. Glucose formed was estimated after PAVY-SUTO-KUMAGAWA's method.

II. Optimum pH for the amylase in digestive diverticula.

In Table 4 the optimum pH for the digestion of glycogen by the extract of digestive diverticula is indicated.

TABLE   4.

Flask	pH of buffer solution	pH of the mixture	Glucose in per cent.
1	3.68	3.70	0.0083
2	4.65	4.68	0.0125
3	5.30	5.28	0.0986
4	5.90	5.90	0.1230
5	6.46	6.45	0.1613
6	6.80	6.80	0.1204
7	7.38	7.37	0.0967
8	7.91	7.88	0.0343
9	8.57	8.54	0.0215
10	9.20	9.12	0.0106

To each of the mixtures of 10 cc. extract + 25 cc. 2% glycogen + 5 cc. 2% NaCl was added 10 cc. of varying buffer solutions, giving 10 different values of pH, and were incubated in a warm water bath for 12 hours at 37–38°C.   Buffer solutions were prepared after SÖRENSEN's method and the value was determined colorimetrically after mixing the solutions.   Glucose was estimated after PAVY-SUTO-KUMAGAWA's method.

INFLUENCE OF pH ON THE PROTEASE OF THE DIGESTIVE DIVERTICULA.

I.   Casein digestion.

The influence of pH on the digestion of casein by the water extract of digestive diverticula was investigated and the results obtained are shown in Table 5.

TABLE   5.

Flask	pH of buffer solution	pH of the mixture	Nitrogen found in 2 cc. mixture, in mg.
1	1.93	2.5	0.352
2	2.97	3.7	0.450
3	3.69	4.0	0.501
4	4.16	4.3	0.480
5	5.04	4.9	0.425
6	6.08	5.8	0.395
7	6.37	6.1	0.373
8	6.80	6.55	0.432
9	7.55	7.3	0.445
10	8.88	8.5	0.461
11	9.12	8.8	0.438
12	9.40	9.0	0.417

To each of the mixtures of 10 cc. enzyme + 30 cc. 3% casein solution in NaOH giving pH 7 was added 10 cc. of buffer solutions giving varying values of pH, and altogether mixing the solutions the pH of the mixtures were estimated colorimetrically. A little toluol was added to the mixtures. After 24 hours' digestion the amino nitrogen was estimated by VAN SLYKE's method.

II. Gelatine digestion.

The influence of pH on the gelatine digestion has been studied and the results obtained are shown in Table 6.

TABLE 6.

Flask	pH of buffer solution	pH of the mixture	Amino nitrogen in milligram in 2 cc. of the mixture
1	2.27	2.8	0.280
2	3.25	3.4	0.475
3	3.95	4.1	0.523
4	4.95	5.0	0.501
5	5.90	5.8	0.481
6	6.47	6.3	0.483
7	7.20	7.0	0.492
8	7.73	7.5	0.519
9	8.35	8.0	0.536
10	8.80	8.5	0.534
11	9.39	9.0	0.480
12	10.21	9.8	0.435

To each of the mixtures of 5 cc. enzyme + 10 cc. 2.5% gelatine was added 10 cc. of buffer solution of varying value. After mixing the solutions, pH of the mixture was estimated colorimetrically. After being incubated for 36 hours at 37–38°C, amino nitrogen was estimated after VAN SLYKE's method.

DEMONSTRATION OF BUTYRASE IN CRYSTALLINE STYLE.

To each of 5 test tubes which are labelled A, B, C, D and E were added the solutions as in the manner shown in Table 7. To A, B, C and D were added brom cresol purple and to E brom cresol green as the indicator. The reaction was observed from time to time. The results obtained are shown in Table 7.

The falling of the pH in A and E may have been caused by the

TABLE　7.

Test tube	Saturated trybutyrin	2% Cryst. style	Water	Period of digestion					
				0 m.	1 m.	6 m.	15 m.	50 m.	4 h.
A	2 cc.	1 cc.	—	6.35	6.0	5.7	5.4	—	—
B	2 cc.	1 cc. (boiled)	—	6.35	6.35	6.35	6.3	6.3	6.3
C	—	1 cc.	2 cc.	6.2	6.2	6.2	6.2	6.2	6.2
D	2 cc.	—	1 cc.	6.35	6.35	6.35	6.35	6.35	6.3
E	2 cc.	1 cc.	—	—	—	—	5.4	4.8	4.5

formation of butyric acid from the trybutyrin in the presence of butyrase.

DISCUSSSON OF THE RESULTS.

The presence of amylase in crystalline style was first demonstrated by COUPIN in *Cardium* (1900, cited by YONGE). Both MITRA (1901) and NELSON (1918) in *Anodonta,* and YONGE in *Mya* also demonstrated its presence in the crystalline style. Recently YONGE (1926) studied in detail the nature of the amylase from the crystalline style of *Ostrea edulis,* and determined the optimum pH at 5.9. In my experiment, as is shown in Table 3, the optimum pH for the amylase from the crystalline style of *Ostrea circumpicta* is 6.0. These results practically agree with that of *Ostrea edulis* found by YONGE. In the case of amylase from digestive diverticula YONGE pointed out the optimum pH to be at 5.5 which is somewhat lower than that found in the crystalline style. On the contrary, in my experiment I found the optimum pH of glycogen digestion to be 6.4, that being slightly higher than that found in the crystalline style. It is difficult to understand the causes of the discrepancy between the results just mentioned, but it may be due to the difference of the substrate used, that is, starch by YONGE and glycogen by the present writer. What is more important in this research is the presence of butyrase and calcified milk clotting activity in the crystalline style, as is shown in Tables 2 and 7.

SUMMARY.

1.   The distributions of amylase and milk clotting enzyme in the body of *Ostrea circumpicta* were determined.

2.   Several enzymes were detected in the crystalline style, in gastric juice and in the extract of digestive diverticula.

3.   The optimum pH for the amylase from crystalline style is 6.0 and for that from digestive diverticula is 6.4.

4.   Milk clotting enzyme and butyrase are demonstrated in the crystalline style.

5.   The optimal pH for protease obtained from digestive divert cula for the digestion of gelatine and casein were investigated. The optimal pH for casein digestion is 4.0 and 8.5 and that for gelatine digestion is 4.1 and 8–8.5.

LITERATURE.

1) BERKELEY, C. 1923.. On the crystalline style as a possible factor in the anaerobic respiration of certain marine mollusks. Journ. Exp. Zoöl., Vol. 37, p. 477.

2) BODANSKY, M. and ROSE, W. C. 1922.   The digestive enzymes of Coelenterates. Amer. Journ. Physiol., Vol. 62, p. 473.

3) BODANSKY, M. 1923.   Further observations on digestion in Coelenterates. Amer. Journ. Physiol. Vol. 67, p. 547.

4) CLARK, W. M. 1920.   Determination of hydrogen ions.

5) DAKIN, W. J. 1909.   *Pecten.* Liverpool Marine Biol. Comm. Memoirs, 17.

6) EDMONDSON, C. H. 1920.   The reformation of the crystalline style in *Mya arenaria* after extraction. Journ. Exp. Zoöl., Vol. 30, p. 259.

7) FREDERICQ, L. 1878.   La digestion des matières albuminoïdes chez quelques invertébrés. Arch. Zoöl. Expér., T. 7, p. 391.

8) JORDAN, H. 1913.   Vergleichende Physiologie wirbelloser Tiere.

9) HUNT, O. D. 1925.   The food of the bottom fauna of the Plymouth fishing grounds. Journ. Marine Biol. Assoc., N. S., Vol. 13, p. 560.

10) MACKINTOSH, N. A. 1925.   The crystalline style in gastropods Q. J. M. S., Vol. 69, p. 317.

11) MARTIN, G. W. 1923.   Food of the oyster, Bot. Gaz., Vol. 75, p. 143.

12) MITRA, S. B. 1901.   The crystalline style of Lamellibranchia. Q. J. M. S., Vol. 44, p. 591.

13) NELSON, T. C. 1925.   Recent contributions to the knowledge of the crystalline style of Lamellibranchs. Biol. Bull., Vol. 19, p. 86.

14) NELSON, T, C. 1918.   On the origin, nature, and function of the crystalline style of Lamellibranchs. Journ. Morph., Vol. 31, p. 53.

15) OPPENHEIMER, C. und CHUN. 1926.   Die Fermente und ihre Wirkungen.

16) ROAF, H. E. 1908. The hydrolytic enzymes of invertebrates. Biochem. Journ., Vol. 3, p. 182.

17) RONA, P. 1926. Praktikum der physiologischen Chemie. Erster Teil. Ferment-methoden.

18) SAWANO, E. 1928. On the digestive enzymes of *Caudina chilensis* (J. MÜLLER), Sci. Rep., Tôhoku Imp. Univ., Fourth Ser., Biol., Vol. 3, p. 206.

19) SHINODA, O. 1928. Über die eiweisslösenden Enzyme im Magensafte von *Astacus*. Zeitschr. f. vergl. Physiol., Bd. 7, S. 323.

20) YONGE, C. M. 1924. The mechanism of feeding, digestion, and assimilation in the Lamellibranch *Mya*. Brit. Jour. Exper. Biol., Vol. 1, p. 15.

21) YONGE, C. M. 1924. The mechanism of feeding, digestion, and assimilation in *Nephrops norvegicus*. Brit. Journ. Exper. Biol., Vol. 1, p. 343.

22) YONGE, C, M. 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. Journ. marine Biol. Assoc., N. S., Vol. 14, p. 295.

Statistical Study on *Caudina chilensis* (J. Müller).[1]

BY

TOSHIHIKO YAMANOUCHI.

(Zoological Laboratory, College of Science, Kyôto Imperial University).

1. *The body weight and the body length.*
2. *The body length.*
3. *The body weight and the integument.*
4. *The body weight and the content of the alimentary canal.*

The relation between the body length and the mass of the body is discussed in detail by PRZIBRAM (1922 pp. 42–49); he proposed the formula $y = k.\sqrt[3]{x}$ (where y represents the length in cms., x the weight or the volume) as generally applicable for representing the above relation. CROZIER (1916) gave the function representing the relation between the weight and the length in *Stichopus moebii*, such that

$$\text{Weight in grams} = (0.034\pm) \times (\text{length in cms})^3$$

and stated that such a type of relation is also true for other species. Recently NOMURA (1928) found in the bivalve *Cythera (Meretrix)* the formula $w = ka^{3.00}$ where w denotes the body weight, a the height of the shell, and k the constant, as one case of the general formula $w = ka^x$ (where x is specific constant) proposed by him.

The specimens used in this study were collected at Moura and at Kugurisaka near the Marine Biological Station. The body length was meassured with a divider and read to millimeters, and the body weight with a balance sensitive to 1 centigram. In the following tables, the mean values were calculated from the correlation tables made from the observed values.

1. THE BODY WEIGHT AND THE BODY LENGTH.

In the computation of the measured numbers, the contents of the alimentary canal, composed of ingested sand, are of great importance.

---

[1] A contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

The weight of the content sometimes reaches 50% of the total body weight. The contents of the alimentary canal of freshly collected animals were more than 25% ± of the total body weight, but this content decreases gradually by continual egestion when the animal is brought out of the sand. For this reason the relation between the body weight and the body length was considered in two ways, first with the total body weight (the body weight with sand) and secondly with the body weight without sand.

a) *The body weight with sand (Ws) and the body length (L).* The

TABLE 1.

Body weight with sand and body length. 159 animals.

Body weight with sand Ws in grams	Number of animals	Average body length observed L in cms.	Body length calculated L' in cms.	Difference $L - L'$ in cms.
3.1	18	4.18	4.49 ⎫	−0.31
6.5	8	5.44	5.75 ⎪	−0.31
9.9	6	7.15	6.61 ⎪	+0.54
13.3	5	6.80	7.30 ⎪	−0.50
16.7	10	8.10	7.87 ⎬ (1)	+0.23
20.1	5	7.90	8.39 ⎪	−0.49
23.5	5	9.60	8.82 ⎪	+0.78
26.9	5	9.50	9.23 ⎪	+0.27
30.3	2	8.90	9.60 ⎭	−0.70
33.7	10	11.50	11.60 ⎫	−0.10
37.1	5	11.80	11.97 ⎪	−0.17
40.5	6	12.48	12.33 ⎪	+0.15
43.9	8	11.96	12.66 ⎪	−0.70
47.3	8	13.35	12.98 ⎪	+0.37
50.7	9	13.73	13.29 ⎪	+0.44
54.1	10	13.65	13.58 ⎪	+0.07
57.5	9	13.84	13.86 ⎪	−0.02
60.9	3	13.40	14.12 ⎬ (2)	−0.72
64.3	8	13.59	14.38 ⎪	−0.79
67.7	5	14.60	14.63 ⎪	−0.03
71.1	3	14.90	14.87 ⎪	+0.03
74.5	3	15.40	15.11 ⎪	+0.29
77.9	3	15.40	15.33 ⎪	+0.07
81.3	2	15.40	15.55 ⎪	−0.15
84.7	1	15.90	15.77 ⎪	+0.13
88.1	1	15.40	15.97 ⎪	−0.57
91.5	1	15.40	16.18 ⎭	−0.78

(1) Calculated from $L = (3.335 - 0.255) Ws^{\frac{1}{3}}$

(2) Calculated from $L = (3.335 + 0.255) Ws^{\frac{1}{3}}$

formula for determining the body length for a given body weight is as follows :

$$L = (3.335 \mp 0.255)Ws^{\frac{1}{3}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where L is body length in cms. and Ws body weight with sand in grams, and for the animals of 0-32.00 grams the upper sign, and for the animals of more than 32.01 grams, the lower sign should be taken, or

$$L = 3.08\, Ws^{\frac{1}{3}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$L = 3.59\, Ws^{\frac{1}{3}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The observed and the calculated values are plotted in text figure 1 (Table 1).
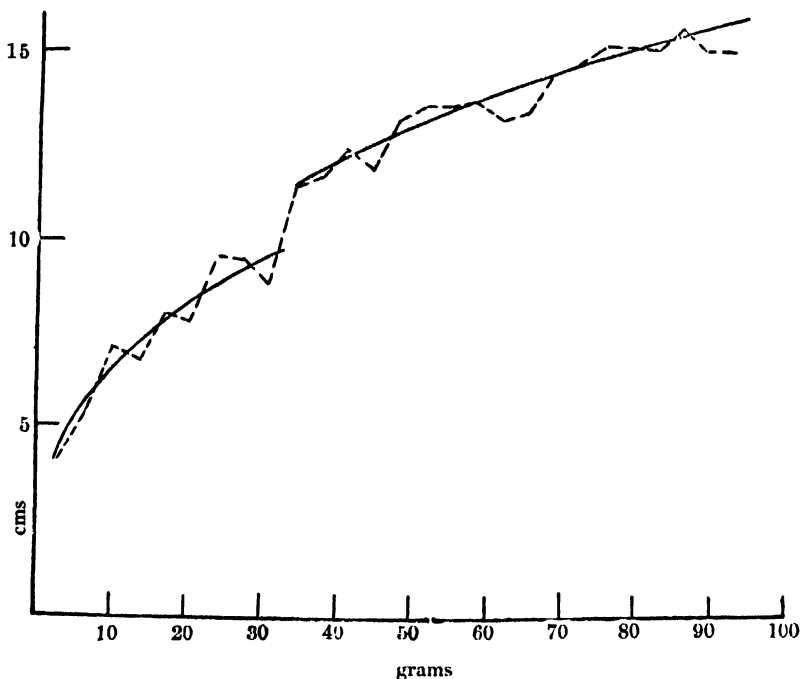
Fig. 1. The ordinate indicates the body length and the abscissa the body weight with sand. The full line indicates the calculated value and the dotted line the observed value.

 b) *The body weight without sand* (W) *and the body length* (L). From the total body weight the weight of the content in the alimentary canal was subtracted and the remaining weight was designated as the body weight without sand. From the data given in Table 2, the relation of L to the given body weight is expressed by the following equation :

$$L = (3.70 \mp 0.28) W^{\frac{1}{3}} \quad \dots\dots\dots\dots\dots\dots\dots (4)$$

TABLE 2.

Body weight without sand and body length. 273 animals.

Body weight without sand W in grams	Number of animals	Average body length observed L in cms.	Body length calculated L′ in cms.	Difference L — L′ in cms.
2.1	33	4.05	4.38 ⎫	—0.33
4.7	20	6.07	5.73 ⎪	+0.34
7.3	11	6.85	6.63 ⎬ (1)	+0.22
9.9	19	7.61	7.34 ⎪	+0.27
12.5	11	8.31	7.94 ⎭	+0.37
15.1	6	9.90	9.84 ⎫	+0.06
17.7	18	10.04	10.38	—0.34
20.3	10	11.20	10.87	+0.33
22.9	9	11.57	11.31	+0.26
25.5	12	11.98	11.72	+0.26
28.1	13	12.44	12.11	+0.33
30.7	16	12.62	12.47	+0.15
33.3	19	13.08	12.81	+0.27
35.9	13	13.25	13.14	+0.11
38.5	17	13.19	13.45 ⎬ (2)	—0.26
41.1	14	13.33	13.75	—0.42
43.7	8	13.46	14.03	—0.57
46.3	6	14.07	14.30	—0.23
48.9	6	14.57	14.57	0.00
51.5	5	14.60	14.82	—0.22
54.1	2	15.40	15.06	+0.34
59.3	3	15.57	15.53	+0.04
64.5	1	15.40	15.97	—0.57
67.1	1	15.40	16.19 ⎭	—0.79

(1) Calculated from $L = (3.70 - 0.28) W^{\frac{1}{3}}$

(2) Calculated from $L = (3.70 + 0.28) W^{\frac{1}{3}}$

where L is body length in cms. and W body weight without sand in grams, and for the animals of 0—13.30 the upper sign, and for the animals of more than 13.31, the lower sign should be taken.
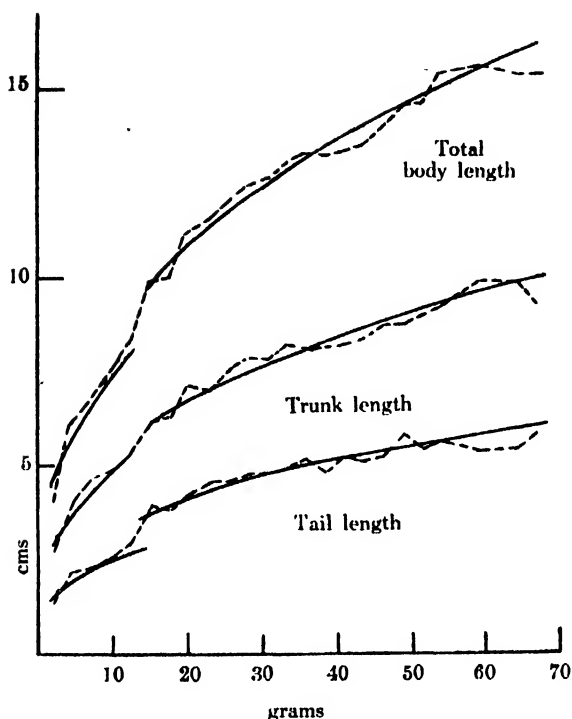
Fig. 2. The ordinate indicates the body length, the abscissa the body weight without sand.

c) *The body weight without sand* (W) *and the lengths of the trunk* (Ltr) *and the tail* (Lta). The posterior part of the body with well developed cloacal muscles is called the tail and the remaining anterior part the trunk. The lengths of the trunk and the tail for the given body weight (Tables 3, 4) are determined by the equations:

$$Ltr = (2.38 \mp 0.10) W^{\frac{1}{3}} \quad \dots\dots\dots\dots\dots\dots\dots (5)$$

$$Lta = (1.34 \mp 0.17) W^{\frac{1}{3}} \quad \dots\dots\dots\dots\dots \dots (6)$$

where Ltr and Lta are trunk and tail lengths in cms. and W body weight without sand in grams; for the animals of 0-13.30 grams the upper sign, and for the animals of more than 13.31 grams the lower sign should be taken. The observed and the calculated values are plotted in figure 2.

TABLE 3.

Body weight without sand and the length of the trunk. 274 animals.

Body weight without sand W in grams	Number of animals	Trunk length observed Ltr in cms.	Trunk length calculated Ltr' in cms.	Difference $Ltr - Ltr'$ in cms.
2.1	33	2.78	2.92	−0.14
4.7	20	4.01	3.84	+0.17
7.3	11	4.64	4.42 (1)	+0.23
9.9	18	4.93	4.90	+0.03
12.5	11	5.31	5.29	+0.02
15.1	6	6.15	6.12	+0.03
17.7	18	6.28	6.46	−0.18
20.3	11	7.15	6.77	+0.38
22.9	9	7.07	7.04	+0.03
25.5	12	7.53	7.30	+0.23
28.1	13	7.77	7.54	+0.23
30.7	16	7.87	7.77	+0.12
33.3	19	8.19	7.98	+0.21
35.9	14	8.14	8.19	−0.05
38.5	17	8.29	8.37 (2)	−0.08
41.1	14	8.18	8.56	−0.38
43.7	8	8.36	8.73	−0.37
46.3	6	8.75	8.91	−0.16
48.9	6	8.80	9.07	−0.27
51.5	5	9.00	9.23	−0.23
54.1	2	9.30	9.38	−0.08
59.3	3	9.90	9.67	+0.23
64.5	1	9.90	9.95	−0.05
67.1	1	9.30	10.08	−0.78

(1) Calculated by $Ltr = (2.38 - 0.10) W^{\frac{1}{3}}$

(2) Calculated by $Ltr = (2.38 + 0.10) W^{\frac{1}{3}}$

TABLE 4.

Body weight without sand and the length of the tail. 272 animals.

Body weight without sand W in grams	Number of animals	Tail length observed Lta in cms.	Tail length calculated Lta' in cms.	Difference $Lta - Lta'$ in cms.
2.1	33	1.35	1.50	−0.15
4.7	20	2.15	1.96	+0.19
7.3	11	2.26	2.27 (1)	−0.01
9.9	18	3.60	2.51	+0.09
12.5	11	2.95	2.71	+0.24

(1) Calculated by $Lta = (1.34 - 0.17) W^{\frac{1}{3}}$

Body weight without sand W in grams	Number of animals	Tail length observed Lta in cms.	Tail length calculated Lta' in cms.	Difference $Lta - Lta'$ in cms.
15.1	6	3.90	3.72	+0.18
17.7	18	3.81	3.93	—0.12
20.3	10	4.16	4.12	+0.04
22.9	9	4.52	4.29	+0.23
25.5	12	4.55	4.44	+0.11
28.1	13	4.73	4.59	+0.14
30.7	16	4.76	4.73	+0.03
33.3	19	4.82	4.86	—0.04
35.9	13	5.12	4.99	+0.13
38.5	17	4.84	5.10 (2)	—0.26
41.1	14	5.29	5.21	+0.08
43.7	8	5.10	5.32	—0.22
46.3	6	5.23	5.42	—0.19
48.9	6	5.77	5.52	+0.25
51.5	5	5.54	5.62	—0.08
54.1	2	5.70	5.71	—0.01
59.3	3	5.40	5.89	—0.49
64.5	1	5.50	6.06	—0.56
67.1	1	5.90	6.14	—0.24

(2) Calculated by $Lta - (1.34 + 0.17) W^{\frac{3}{3}}$

2. THE BODY LENGTH.

The ratio of the trunk length to the tail length is not the same in all animals. In Table 5 the corresponding lengths of the trunk and

TABLE 5.

Correlation of the body length and the lengths of the trunk and the tail. Number of animals 274.

Total body length in cms.	Number of animals	Trunk length in cms.	Tail length in cms.	$\dfrac{\text{Trunk}}{\text{Trunk}+\text{Tail}} \cdot 100$
3.5	17	2.40	1.12	68.2
4.5	14	3.14	1.43	68.7
5.5	11	3.56	1.84	65.9
6.5	18	4.31	2.20	66.2
7.5	17	4.89	2.60	65.3
8.5	18	5.44	2.95	64.8
9.5	12	6.03	3.45	63.6
10.5	21	6.41	4.10	61.0
11.5	28	6.96	4.44	61.1
12.5	39	7.76	4.73	62.1
13.5	34	8.46	4.97	63.0
14.5	25	9.12	5.29	63.3
15.5	18	9.70	5.68	63.1
16.5	2	10.20	6.20	62.2

the tail are averaged for the given total body length, and in figure 3 the percentage ratio of the trunk and the tail to the total body length is plotted from Table 5.
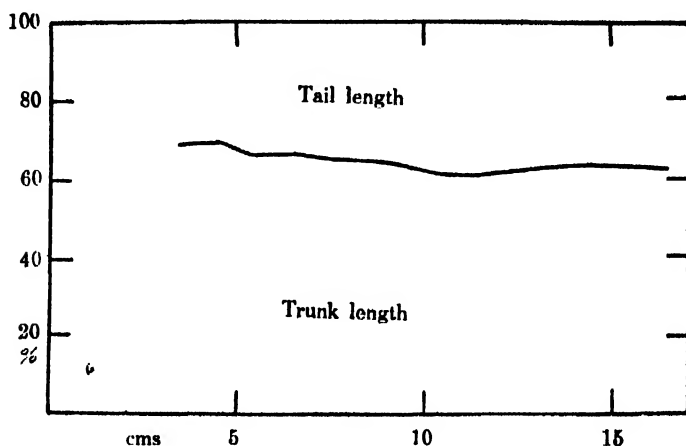
Fig. 3. The abscissa indicates the total body length and the ordinate the percentage ratio of the trunk and the tail.

With the growth of the body, the relative length of the trunk decreased gradually, reaching the minimum at the total length of 10.5 cms., and from this point again increased to the limit of the growth.

3. THE BODY WEIGHT AND THE INTEGUMENT.

The integument means, in this paper, the whole body wall, that is, all of the body except the body fluid, the visceral organ, and the calcareous ring. After finding the body weight, the integument was isolated and weighed to centigrams. The body weight without sand for the given weight of the fresh integument is determined by the following equation,

$$W = 4.69\,I + 1.40 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (7)$$

where W is the body weight without sand in grams and I the fresh weight of the integument in grams (Table 6, Fig. 4).

TABLE 6.

Body weight without sand and the fresh integument. 246 animals.

Fresh integument *I* in grams	Number of animals	Body weight without sand observed *W* in grams	Body weight calculated* *W'* in grams	Difference *W - W'* in grams
0.6	22	4.05	4.21	−0.16
1.6	26	11.48	8.90	+2.58
2.6	11	14.55	13.59	+0.96
3.6	18	15.53	18.28	−2.75
4.6	13	23.45	22.97	+0.48
5.6	26	27.01	27.66	−0.65
6.6	51	33.01	32.35	+0.66
7.6	34	36.82	37.04	−0.22
8.6	23	42.57	41.73	+0.84
9.6	12	48.95	46.42	+2.53
10.6	6	50.00	51.11	−1.11
11.6	3	53.50	55.80	−2.30
13.6	1	57.70	65.18	−7.48

* Calculated from $W = 4.69I + 1.40$
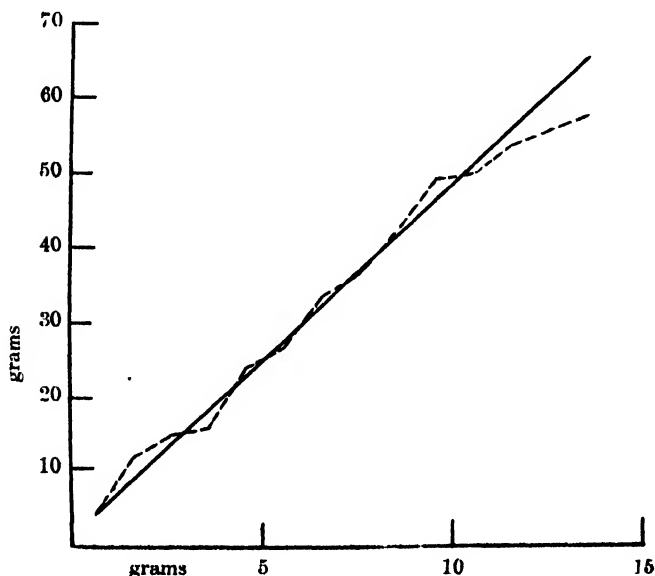
Fig. 4. The ordinate indicates the body weight without sand and abscissa the fresh weight of integument.

For animals of less than 20 grams the equation is not strictly applicable, owing to the rapid change in the growth of the integument.

4. THE BODY WEIGHT AND THE CONTENT OF THE ALIMENTARY CANAL.

The ingested sand was removed from the alimentary canal of the freshly collected animals and weighed after the water was removed by filter paper as well as possible. The weight of the sand for the given total body weight is determined by the equation,

$$S = 0.3217 \, Ws + 1.18 \dots \dots \dots \dots \dots (8)$$

where S is the sand, and Ws the body weight with sand, both expressed in grams (Table 7, Fig 5).

TABLE   7.

Body weight with sand, and sand in the alimentary canal.   212 animals.

Body weight with sand Ws in grams	Number of animals	Sand observed S in grams	Sand calculated* S' in grams	Difference $S - S'$ in grams
4.56	27	1.75	2.65	−0.90
10.76	14	4.31	4.64	−0.33
16.96	16	6.45	6.64	−0.19
23.16	12	7.95	8.63	−0.68
29.36	11	11.63	10.63	+1.00
35.56	21	13.55	12.62	+0.93
41.76	17	15.68	14.61	+1.07
47.96	22	16.99	16.61	+0.38
54.16	24	19.28	18.60	+0.68
60.36	17	19.69	20.60	−0.91
66.56	13	23.30	22.59	+0.89
72.76	8	25.21	24.59	+0.62
78.96	4	23.45	26.58	−3.13
85.16	5	25.85	28.58	−2.73
91.36	1	25.45	30.58	−5.13

*Calculated from $S = 0.3217 \, Ws + 1.18$

From figure 5, it follows that in the animals above 70 grams the content of the sand decreases more than as expected from the body weight. In Fig. 10 is plotted the sand content for the given body weight without sand.
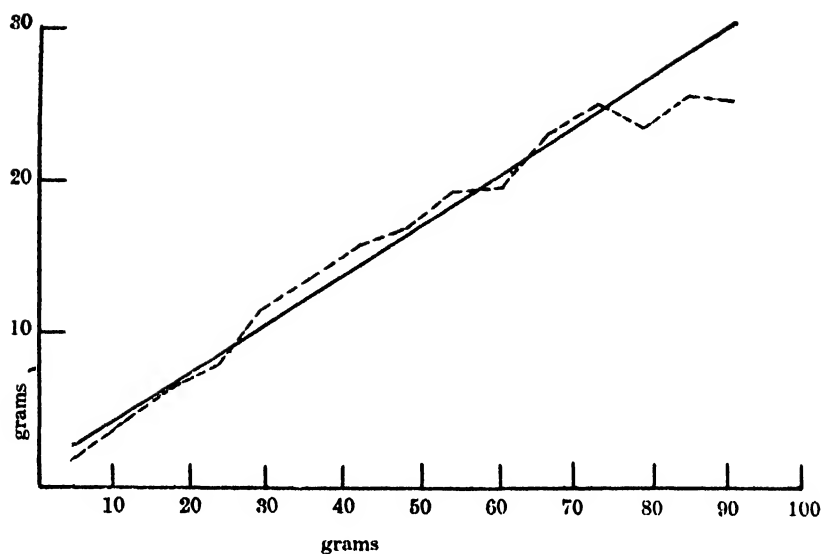
Fig. 5. The ordinate indicates the content of the alimentary canal and the abscissa the body weight with sand.
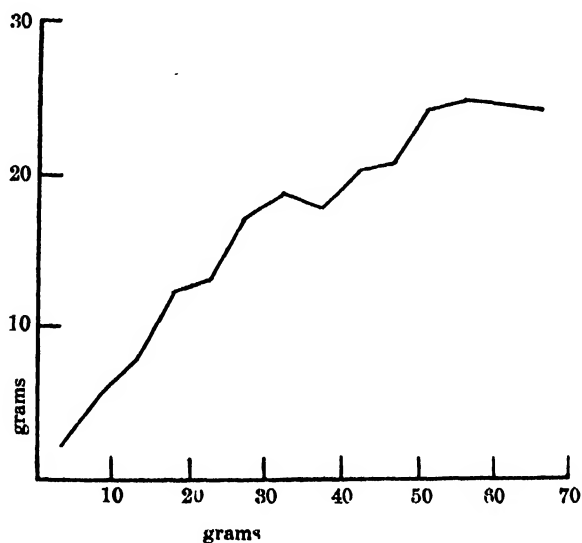
Fig. 6. The ordinate indicates the content of the alimentary canal and the abscissa the body weight without sand.

5. GENERAL REMARKS.

Notwithstanding the diverse changes in the body length owing to the various internal and external conditions, the relation between the body weight and the length of the body or part of it is determined by the simple formula $L = k \cdot \sqrt[3]{W}$ under the condition that the animal is brought out of the sand and is in the definite tonic contraction of all the body musculatures. Transforming the equation (2) $L = 3.08\, Ws^{\frac{1}{3}}$, we have

$$Ws = 0.03422\, L^3 \dots\dots\dots\dots\dots\dots\dots\dots (9)$$

which is nothing but the equation $w = (0.034 \pm) l^3$ given by CROZIER in *Stichopus* as previously mentioned.

$$L = (3.70 \mp 0.28) W^{\frac{1}{3}} \dots\dots \dots\dots\dots\dots (4)$$

$$Ltr = (2.38 \mp 0.10) W^{\frac{1}{3}} \dots\dots\dots\dots\dots\dots (5)$$

$$Lta = (1.34 \mp 0.17) W^{\frac{1}{3}} \dots\dots\dots\dots\dots (6)$$

when (5) + (6), we have $L = Ltr + Lta = (3.72 \mp 0.27) W^{\frac{1}{3}} \dots\dots (10)$

which is almost equal to the equation (4). NOMURA (1928 p. 113) found also such a relation among the constants in *Cythera*.

A sudden increase in length occurred about 30 grams (Fig. 1) and between 10 and 20 grams (Fig. 2). Such discontinuity seems to be dependent upon the change in growth rate; and the discrepancy is greater in the tail than in the trunk, as is clearly indicated by the constant in (5) and in (6). The relative length of the tail to the trunk increased with the increase of the total length and reached the maximal ratio at 10.5 cms. of the body length, which corresponds to the length at which the discontinuity occurred (Figs. 1, 2, 3). The fresh weight of the integument increased with acceleration in the animals below 15 grams and reached the maximum at 15.5 grams (without sand). From these facts, it may well be supposed that some changes in growth rate and also in general metabolism occurred in the animals of about 30 grams (with sand) or of 10–20 grams (without sand). In the older animals, the content of the alimentary canal decreases parallel to the regressive activity (Fig. 5, 6).

6. SUMMARY.

1. When the animal is brought into the air, we can measure the body length under the same conditions (under the conditions that the animal has practically the same *physiological length*). The body length or its parts are expressed for the given body weight without sand by the equation,

$$L = kW^{\frac{1}{3}}$$

where L is body length in cms., W body weight without sand in grams and k the constant; for the given body weight with sand by the equation,

$$L = kWs^{\frac{1}{3}}$$

where Ws is body weight with sand in grams, and L total body length in cms.

2. The relative length of the tail to the trunk becomes maximal at the total body length of 10.5 cms.

3. The relation of the body weight for the given weight of fresh integument is expressed by

$$W = kI + k'$$

where I is the fresh weight of integument in grams and k, k' the constants.

4. The contents of the alimentary canal are chiefly composed of ingested sand. We can calculate approximately this sand content from the body weight by the equation

$$S = kWs + k'$$

where S is sand content in grams. The sand content decreases in the older animals in comparison with the younger animals.

I desire to express my indebtness to Prof. HATAI for his kind direction and encouragement. I am also indebted to Prof. E. NOMURA for his kindness in giving me valuable criticism.

LITERATURE CITED.

1) CROZIER, W. J. 1916. The rhythmic pulsation of the cloaca of Holothurians. Exp. Zool. Vol. 20, pp. 297-353.
2) NOMURA, E. 1928. On the relation between Weight and Dimension in the Bivalves, *Tapes philippinarum* ADS. & RVE. and *Cythera meretrix* LINN. Science Reports, Tôhoku Imp. Univ., Biology, Vol. III, p. 113.
3) PRZIBRAM. H. 1922. Form und Formel im Tierreiche. Leipzig und Wien. pp. 42-49.

TABLE 8.

Body weight and body length.
Specimens collected at Moura in 1926.
*denotes specimens collected at Kugurisaka in 1927.
Ws : body weight with sand in grams.
L : body length in cms.

Ws	L	Ws	L	Ws	L	Ws	L
1.46	3.9*	15.76	7.2	39.26	13.2	56.54	14.9
1.56	3.0*	16.12	7.6			56.79	13.1
1.56	3.9*	16.14	9.0	41.20	12.6	56.89	12.6
1.82	3.7*	17.14	7.9	41.70	10.9	57.37	13.1
1.91	3.8*	17.80	8.1	41.85	12.7	57.40	15.7
2.00	3.7*	18.12	7.1	43.05	11.0	58.13	13.3
2.57	3.6*	19.74	6.5	43.54	11.8	58.77	13.0
2.63	3.6*	19.96	8.4	43.51	10.6	59.14	14.8
2.74	3.5*			44.17	12.6	59.43	14.7
2.85	4.2*	20.37	7.3	44.89	12.8		
3.22	4.3*	21.64	8.9	45.19	12.9	61.07	13.1
3.35	4.2*	21.75	8.3	45.50	12.8	62.10	12.7
3.36	4.1*	23.96	9.2	45.73	11.1	63.77	13.0
3.49	4.2*	24.53	9.7	46.21	13.5	64.04	12.5
3.79	5.4	24.66	9.3	47.08	13.7	64.08	14.7
3.85	5.6	24.85	9.8	47.17	12.6	64.16	12.7
4.17	5.1*	25.18	10.5	47.84	12.4	64.89	14.9
4.18	5.1*	26.23	10.2	48.00	12.6	65.27	14.6
4.84	4.7*	26.94	9.8	48.11	15.6	65.60	15.1
5.29	4.9	27.43	8.8	48.29	14.1	65.94	11.5
5.54	4.8*	28.00	8.3	48.80	12.6	66.26	14.7
5.67	6.7	28.57	10.9	49.50	13.5	66.82	15.1
6.48	6.6					67.24	12.5
7.25	6.2	30.46	9.3	50.12	12.3	67.74	16.0
7.29	6.3	30.69	8.7	50.36	13.7	68.57	14.2
7.82	7.1	32.05	12.0	50.62	13.6		
8.44	7.0	32.69	10.2	50.64	15.8	70.91	15.2
9.39	7.4	33.30	12.8	52.14	14.2	72.32	15.7
9.89	6.0	33.77	10.4	52.29	13.4	72.61	13.9
9.90	6.8	33.83	13.6	52.3·	13.2	73.65	14.6
		34.00	10.3	52.37	14.8	75.44	16.5
10.50	7.9	34.29	10.4	52.51	14.7	75.85	15.0
11.43	7.3	34.32	10.7	53.36	13.9	76.52	15.0
11.62	6.6	34.86	11.1	53.46	12.1	76.66	15.3
11.81	7.6	35.15	13.2	53.71	13.3	76.71	15.2
11.88	6.5	35.67	10.9	53.75	13.7		
13.87	6.5	36.69	10.9	54.00	13.5	82.70	15.2
14.78	7.3	37.41	12.4	54.61	13.6	82.79	15.0
15.11	7.9	37.93	12.5	54.77	13.8	85.19	15.9
15.25	8.7	38.30	12.6	54.79	14.1	86.56	15.4
15.49	8.2	38.99	13.6	54.94	14.4		
15.63	8.0	39.19	12.2	56.41	13.9	93.01	15.1

TABLE 9.

Body weight and body length.
Specimens collected at Moura in 1927.
*denotes specimens collected at Kugurisaka in 1927.
W : body weight without sand in grams.
L : body length in cms.

W	L	W	L	W	L	W	L	W	L
0.85	3.0*			12.52	8.4	24.01	11.1	32.22	14.9
1.00	3.7*	5.02	6.8	12.58	8.6	24.07	13.6	32.28	13.7
1.01	3.9*	5.07	5.5	13.29	8.3	24.15	10.5	32.42	14.8
1.05	3.1*	5.15	7.9	13.48	6.5	24.62	11.4	32.55	13.9
1.06	3.6*	5.50	5.1	14.40	8.9			32.87	14.3
1.06	3.9*	5.63	6.0	14.92	10.5	25.08	11.2	32.91	11.7
1.21	3.1*	5.63	7.0	14.96	10.9	25.26	11.5	33.04	11.0
1.22	3.8*	5.74	6.9			25.96	12.6	33.20	13.5
1.24	3.6*	6.01	6.6	15.57	9.3	26.03	12.6	33.57	14.1
1.28	3.7	6.28	5.6	16.16	10.6	26.10	12.7	33.58	12.0
1.33	3.7*	6.52	7.4	16.26	9.2	26.16	10.6	33.67	14.1
1.42	3.5*	6.76	7.2	16.44	10.3	26.25	11.6	33.95	12.9
1.50	4.1*	7.23	5.7	16.65	9.2	26.31	12.5	34.03	12.4
1.53	3.2*	7.65	7.3	16.77	9.3	26.59	12.0	34.19	13.3
1.54	4.2*	7.67	6.9	16.63	8.3	26.60	13.6	34.21	13.0
1.60	3.2*	7.74	6.5	17.04	9.3	26.75	12.4	34.22	13.2
1.61	3.8*	8.00	6.6	17.19	9.8	27.09	9.4	34.24	11.9
1.68	3.5*	8.24	7.6	17.18	10.1	27.62	13.5	34.34	11.0
1.68	4.2*	8.43	8.2	17.47	10.4	27.76	11.6	34.39	11.7
1.68	4.2*	8.84	8.0	17.49	10.2	28.06	10.0	34.77	12.6
1.85	4.2*	8.84	7.6	17.53	10.7	28.15	13.7		
1.91	4.2*	8.85	7.9	17.88	10.9	28.41	11.8	35.01	15.6
1.99	3.9*	8.93	7.3	17.97	9.7	28.52	14.4	35.05	12.5
2.11	4.1	8.97	8.7	18.49	9.7	28.64	12.6	35.05	13.9
2.32	5.1*	9.18	6.5	18.61	9.8	28.75	12.9	35.29	11.7
2.37	5.1*	9.21	9.0	18.68	11.1	28.97	12.2	35.69	13.1
2.64	4.3	9.31	6.6	18.70	10.2	29.04	13.6	35.94	13.4
2.85	4.7*	9.36	7.1	16.92	8.8	29.22	11.1	36.00	12.3
2.88	5.6	9.66	6.4	18.97	13.2	29.24	15.8	36.08	13.0
2.89	4.5*	9.85	7.2	19.06	10.4	29.40	14.1	36.20	13.2
2.99	5.4			19.13	11.1	29.42	11.6	36.33	14.9
3.28	4.8*	10.01	7.6	19.18	10.3	29.48	13.8	36.57	12.6
3.29	6.5	10.13	8.1	19.65	10.3	29.73	11.1	37.02	13.5
3.47	6.6	10.14	7.5			29.87	12.8	37.20	12.3
3.58	4.9	10.30	8.1	20.22	8.7	29.88	12.9	37.29	13.4
3.82	5.5	10.71	7.2	20.27	12.0			37.33	12.4
3.87	4.5	10.71	7.2	20.92	12.2	30.25	11.5	37.33	12.8
4.03	6.3	10.53	7.9	21.01	11.3	30.30	14.8	37.99	12.9
4.05	4.5	10.85	7.3	21.08	14.0	30.51	14.7	36.10	13.6
4.10	6.7	11.36	8.3	21.19	10.9	31.03	12.1	38.31	14.2
4.13	5.2	11.60	8.7	21.89	12.1	31.43	9.4	38.42	13.0
4.29	7.1	11.65	8.4	22.06	13.3	31.60	13.7	38.43	15.1
4.55	6.2	12.20	8.1	22.15	11.1	31.74	13.0	38.45	13.0
4.63	6.3	12.23	9.3	22.74	10.7	31.78	13.3	38.82	14.2
4.65	6.7	12.41	8.0	23.81	10.2	31.90	10.6	39.07	14.1
4.77	5.8	12.46	8.5	23.86	10.9	31.91	13.5	39.10	11.6

W	L	W	L	W	L	W	L	W	L
39.12	12.2	41.41	14.7	43.45	13.3	47.95	13.7	51.36	13.6
39.15	13.1	41.45	12.4	43.77	11.7	48.02	14.2	53.52	15.0
39.39	12.3	41.05	14.4	44.89	13.9	48.15	16.5	54.32	15.3
39.58	12.3	41.51	14.9	44.98	16.0	48.31	13.9		
39.85	12.7	41.90	12.6			49.53	15.1	58.02	15.9
39.92	12.7	42.15	14.2	45.00	15.7	49.57	14.5	58.20	15.0
		42.19	13.5	45.32	11.5			58.33	15.2
40.10	13.7	42.40	12.7	46.09	15.2	50.22	14.6		
40.20	11.3	42.70	14.6	46.26	15.1	50.68	15.0	63.34	15.4
40.40	15.7	42.96	13.1	46.35	15.0	50.77	14.6		
40.46	12.5	43.38	12.5	47.18	11.3	50.89	15.2	67.27	15.1
41.25	12.9								

TABLE 10.

Body length.

Specimens collected at Moura in 1926.

*denotes specimens collected at Kugurisaka in 1927.

Ltr : trunk length in cms.

Lta : tail length in cms.

L : total body length (Ltr Lta) in cms.

Ltr	Lta	L	Ltr	Lta	L	Ltr	Lta	L	Ltr	Lta	L
2.0	1.0	3.0*	3.0	1.5	4.5*	4.1	2.5	6.6	5.1	3.0	8.1
2.1	1.0	3.1*	3.0	1.7	4.7*	4.1	2.6	6.7	5.2	2.9	8.1
2.0	1.1	3.1*	3.2	1.6	4.8*	4.4	2.4	6.6	5.0	3.1	8.1
2.0	1.2	3.2*	3.5	1.4	4.9	4.1	2.6	6.7	5.4	2.8	8.2
2.3	0.9	3.2*				4.6	2.2	6.8	5.6	2.7	8.3
2.5	1.0	3.5*	3.2	1.9	5.1	4.2	2.7	6.9	5.7	2.6	8.3
2.3	1.2	3.5*	3.3	1.8	5.1	4.5	2.4	6.9	5.6	2.7	8.3
2.4	1.2	3.6*	3.2	1.9	5.1				5.8	2.6	8.4
2.4	1.2	3.6*	3.4	1.8	5.2	4.4	2.6	7.0	5.3	3.1	8.4
2.4	1.3	3.7*	3.8	2.0	5.3	4.7	2.3	7.0	5.2	3.3	8.5
2.3	1.4	3.7*	3.4	2.0	5.4	4.4	2.7	7.1	5.1	3.5	8.6
2.7	1.0	3.7	3.8	1.7	5.5	4.5	2.6	7.1	5.5	3.2	8.7
2.7	1.1	3.8*	3.8	1.8	5.6	4.5	2.7	7.2	5.6	3.1	8.7
2.6	1.2	3.8*	3.9	1.7	5.6	4.7	2.5	7.2	5.7	3.0	8.7
2.6	1.3	3.9*	3.7	2.0	5.7	4.9	2.4	7.3	6.3	2.5	8.8
2.6	1.3	3.9*	3.7	2.1	5.8	4.5	2.8	7.3	5.5	3.4	8.9
2.6	1.3	3.9*				4.9	2.4	7.3			
			4.1	1.9	6.0	5.3	2.1	7.4	6.1	2.9	9.0
2.6	1.5	4.1*	4.3	1.9	6.2	4.8	2.7	7.5	5.6	3.6	9.2
2.8	1.3	4.1	4.0	2.3	6.3	4.9	2.7	7.6	5.7	3.6	9.3
2.5	1.7	4.2*	4.6	1.7	6.3	5.0	2.6	7.6	6.1	3.2	9.3
3.1	1.1	4.2*	4.5	1.9	6.4	5.7	2.2	7.9	6.0	3.3	9.3
3.1	1.1	4.2*	4.4	2.1	6.5	5.0	2.9	7.9	5.5	3.8	9.3
2.8	1.4	4.2*	4.0	2.5	6.5	5.4	2.5	7.9	5.9	3.5	9.4
3.0	1.3	4.3*	4.6	1.9	6.5	4.5	3.1	7.6	6.0	3.4	9.4
3.0	1.3	4.5	4.4	2.1	6.5				6.2	3.5	9.7
3.2	1.3	4.5	4.4	2.2	6.6	5.3	2.7	8.0	6.8	2.9	9.7
3.1	1.4	4.5	4.3	2.3	6.6	5.0	3.0	8.0	5.9	3.9	9.8

Ltr	Lta	L	Ltr	Lta	L	Ltr	Lta	L	Ltr	Lta	L
6.3	3.5	9.8	6.6	5.0	11.6	8.0	4.9	12.9	8.7	5.4	14.1
			6.6	5.2	11.7	8.2	4.7	12.9	9.3	4.8	14.1
6.0	4.0	10.0	7.7	4.0	11.7	7.7	5.2	12.9	8.8	5.4	14.2
6.1	4.0	10.1	7.0	4.7	11.7	8.4	4.5	12.9	8.2	6.0	14.2
6.8	3.4	10.2	7.0	4.7	11.7				8.3	5.9	14.2
6.2	4.0	10.2	7.5	4.3	11.8	8.0	5.0	13.0	8.8	5.9	14.2
6.9	3.3	10.2	7.5	4.4	11.9	8.1	4.9	13.0	9.3	5.0	14.3
6.4	3.9	10.3	7.2	4.7	11.9	7.8	5.2	13.0	9.2	5.2	14.4
5.7	4.6	10.3				8.0	5.0	13.0	9.6	4.8	14.4
6.4	3.9	10.3	7.8	4.2	12.0	8.5	4.5	13.0	9.5	5.0	14.5
5.6	4.8	10.4	7.6	4.4	12.0	8.4	4.7	13.1	8.4	6.2	14.6
6.5	3.9	10.4	6.9	5.1	12.0	8.3	4.9	13.1	9.1	5.5	14.6
6.4	4.1	10.5	6.9	5.2	12.1	8.8	4.3	13.1	9.7	4.9	14.6
6.8	3.7	10.5	8.3	3.8	12.1	7.7	5.5	13.2	9.7	5.0	14.7
6.8	3.8	10.6	7.9	4.3	12.2	8.9	4.3	13.2	9.5	5.2	14.7
6.5	4.1	10.6	6.4	5.8	12.2	9.0	4.2	13.2	9.6	5.1	14.7
6.0	4.6	10.6	7.4	4.8	12.2	8.2	5.0	13.2	9.3	5.5	14.8
6.3	4.4	10.7	8.2	4.1	12.3	8.3	5.0	13.3	9.6	5.2	14.8
6.7	4.0	10.7	8.0	4.3	12.3	8.1	5.2	13.3	9.7	5.2	14.9
6.9	4.0	10.9	7.7	4.6	12.3	8.3	5.0	13.3	9.7	5.9	14.9
6.8	4.1	10.9	7.7	4.6	12.3	8.4	5.0	13.4	9.8	5.1	14.9
6.8	4.1	10.9	8.3	4.1	12.4	8.8	4.6	13.4			
6.7	4.2	10.9	7.5	4.9	12.4	8.6	4.9	13.5	9.5	5.5	15.0
6.9	4.1	11.0	7.6	4.8	12.4	8.3	5.2	13.5	9.8	5.2	15.0
7.1	3.9	11.0	7.4	5.0	12.4	9.5	4.0	13.5	9.5	5.5	15.0
6.4	4.7	11.1	7.5	5.0	12.5	7.9	5.7	13.6	9.0	6.0	15.0
7.3	3.8	11.1	7.4	5.1	12.5	7.8	5.8	13.6	9.2	5.9	15.1
6.3	4.8	11.1	8.4	4.1	12.5	8.4	5.2	13.6	8.8	6.3	15.1
7.0	4.1	11.1	8.7	3.8	12.5	8.8	4.8	13.6	8.8	6.3	15.1
6.3	4.8	11.1	6.7	5.8	12.5	9.7	3.9	13.6	9.3	5.8	15.2
7.0	4.1	11.1	7.8	4.8	12.6	8.5	5.2	13.7	10.1	5.1	15.2
6.7	4.5	11.2	7.2	5.4	12.6	8.7	5.0	13.7	10.1	5.1	15.2
6.4	4.9	11.3	7.5	5.1	12.6	7.7	6.0	13.7	10.0	5.2	15.2
6.7	4.6	11.3	8.0	4.6	12.6	8.2	5.5	13.7	9.4	5.9	15.3
6.8	4.5	11.3	8.5	4.1	12.6	9.5	4.3	13.8	9.9	5.5	15.4
7.1	4.3	11.4	8.0	4.7	12.7	8.2	5.7	13.9	9.8	5.8	15.6
7.1	4.4	11.5	7.4	5.3	12.7	8.5	5.4	13.9	9.5	6.2	15.7
7.7	3.8	11.5	8.1	4.6	12.7	8.8	5.1	13.9	9.8	5.9	15.7
7.5	4.0	11.5	8.1	4.6	12.7	8.6	5.3	13.9	10.6	5.3	15.8
6.6	5.0	11.6	7.8	4.9	12.7				10.5	5.4	15.9
7.2	4.0	11.6	7.3	5.5	12.8	9.0	5.0	14.0			
7.8	3.8	11.6	7.8	5.0	12.8	9.0	5.0	14.0	10.0	6.0	16.0
5.6	5.0	11.6	9.2	3.6	12.8	8.6	5.5	14.1	10.1	6.8	16.9
			7.7	5.2	12.9	9.1	5.0	14.1			

TABLE 11.

Body weight without sand (W) and the weight of fresh integument (I), both expressed in grams. Specimens collected at Moura in 1926.

W	I	W	I	W	I	W	I	W	I
1.28	0.29	12.20	1.51	24.15	6.33	33.58	6.34	41.25	7.79
2.11	0.57	12.23	3.00	24.62	5.28	33.67	6.91	41.45	7.67
2.64	0.67	12.52	2.34	24.78	6.28	33.95	5.94	41.51	6.92
2.88	0.87	12.46	3.93			34.19	7.04	41.90	8.07
2.99	0.80	12.58	3.20	25.08	5.54	34.21	5.92	42.06	8.33
3.29	0.89	13.29	2.50	25.26	4.76	34.34	4.97	42.07	7.09
3.47	0.68	13.48	3.81	25.36	5.63	34.39	6.29	42.19	8.52
3.58	0.94	14.46	2.65	25.96	4.84	34.62	6.30	42.15	7.35
3.82	0.85	14.92	3.83	26.10	5.66	34.77	6.73	42.40	10.62
3.87	0.81			26.25	7.78	34.24	4.55	42.96	6.83
4.05	0.72	15.57	4.35	26.16	6.72			43.38	8.15
4.10	1.26	16.16	6.16	26.31	6.56	35.01	6.11	43.45	6.96
4.13	0.59	16.26	3.93	26.60	6.40	35.05	6.78	43.77	10.02
4.57	0.90	16.44	3.77	26.85	6.40	35.05	5.66	44.24	7.37
4.63	1.37	16.63	3.09	27.09	5.74	35.29	6.46	44.98	7.31
4.65	0.90	16.65	3.25	27.62	5.87	35.69	6.84	44.98	7.17
4.77	1.24	16.77	3.04	27.73	6.70	35.87	7.90		
		16.92	3.78	27.76	3.43	35.94	6.21	45.00	9.32
5.02	0.41	17.04	2.89	28.06	5.76	36.00	6.82	46.26	9.07
5.07	0.95	17.18	4.84	28.52	8.38	36.08	8.22	46.35	7.03
5.15	0.88	17.19	3.74	28.64	5.35	36.33	6.91	47.18	10.99
5.50	1.22	17.47	5.15	28.75	6.67	36.49	7.23	47.37	9.37
5.63	0.74	17.49	2.76	28.97	7.38	36.57	7.70	47.95	8.58
5.63	2.04	17.53	5.81	29.04	5.77	37.02	8.55	48.02	8.70
5.74	1.41	17.88	3.30	29.24	5.37	37.04	6.83	48.15	9.03
6.01	0.99	17.96	3.79	29.40	6.67	37.19	8.51	48.31	11.10
6.52	0.92	19.06	4.73	29.42	8.31	37.20	6.52	48.41	8.20
7.23	1.40	19.13	7.06	29.54	9.70	37.29	6.65	48.52	10.24
7.65	1.00	19.18	5.92	29.55	5.29	37.33	7.38	49.53	7.15
7.67	1.28	19.43	4.58	29.73	4.66	37.33	7.91	49.57	9.91
7.71	0.90	18.49	2.49	29.87	6.52	37.99	6.73	49.62	7.42
7.78	1.97	18.61	3.00	29.88	7.30	38.10	6.44		
8.00	1.92	18.67	4.24			38.31	7.67	50.22	8.66
8.43	1.13	18.68	7.05	30.25	6.45	38.42	7.24	50.68	8.58
8.67	1.13	18.70	3.67	30.30	6.54	38.43	9.47	50.77	8.76
8.84	1.74	18.97	7.79	30.51	7.32	38.45	6.99	51.36	9.28
8.85	1.39			31.03	6.41	38.82	6.32	52.69	9.30
8.97	1.45	20.27	5.51	31.43	6.24	39.10	5.80	53.52	9.20
9.18	1.26	21.01	3.23	31.60	6.81	39.15	8.06	54.32	10.54
9.21	1.77	21.19	3.76	31.71	7.45	39.26	6.89	54.84	11.34
9.31	1.46	21.89	8.04	31.74	6.53	39.48	8.35	54.98	11.04
9.36	2.07	22.06	5.68	31.91	6.18	39.71	8.14		
9.66	1.83	22.06	5.38	32.22	7.38	39.71	7.31	55.96	9.70
9.85	3.18	22.15	5.27	32.42	7.94	39.92	8.54	56.21	13.94
		22.15	3.26	32.55	8.80			58.20	9.46
10.01	1.35	23.29	4.64	32.58	7.42	40.10	7.21	58.33	8.60
10.13	1.91	23.81	5.48	32.87	6.31	40.20	7.79	58.78	10.50
10.14	1.86	23.86	5.01	32.91	5.41	40.40	8.52	58.02	11.44
10.30	2.24	23.86	6.93	33.04	8.38	40.46	6.02		
10.53	1.31	23.87	4.18	33.11	7.93	40.74	7.01	63.34	9.11
10.85	1.87	24.07	6.41	33.20	7.78	41.05	7.19		
11.36	2.48	24.01	5.28	33.57	6.58	41.30	8.05		

TABLE 12.

Body weight with sand (Ws) and sand in the alimentary canal (S), both expressed in grams. Specimens collected at Moura in 1926.

* denotes specimens collected in 1927.

** denotes specimens collected at Kugurisaka in 1927.

Ws	S	Ws	S	Ws	S	Ws	S	Ws	S
1.46	0.45**	15.11	6.26	33.83	9.49	48.11	13.10	59.43	18.02
1.56	0.49**	15.25	6.28	34.00	14.35	48.20	14.74	59.86	19.91
1.56	0.50**	15.49	7.06	34.29	16.82	48.29	14.72		
1.79	0.71**	15.63	6.77	34.32	11.58	48.80	20.16	60.16	19.42
1.82	0.49**	15.76	5.05	34.86	10.55	49.50	12.48	61.07	18.11
1.91	0.60**	16.12	7.28	34.88	11.01			61.14	15.39
		16.14	6.93	34.90	12.41	50.36	18.08	61.84	22.58
2.00	1.00**	17.14	6.61	35.15	13.19	50.42	24.44*	62.10	22.25
2.57	1.33**	17.80	5.60	35.67	14.48	50.60	18.89	63.77	27.69
2.63	0.57**	18.12	8.76	36.60	18.81	50.62	21.58	64.04	23.58
2.74	1.32**	18.26	4.87*	37.41	10.66	50.64	21.40	64.16	24.24
2.85	1.31**	18.95	6.20*	37.67	12.14*	52.14	13.83	64.79	16.38
3.22	1.37**	19.74	6.26	37.93	11.62	52.29	16.06	64.89	23.38
3.35	1.44**	19.96	8.31	38.30	12.34	52.30	18.08	65.27	22.54
3.36	1.86**			38.42	18.99	52.37	19.95	65.60	19.34
3.49	1.81**	20.15	6.33*	38.99	12.39	52.51	21.94	65.94	20.59
3.79	1.54	20.37	9.82	39.19	18.27	52.91	20.32	66.26	26.22
3.85	0.97	20.66	8.32*	39.26	10.29	53.09	16.60	66.82	28.39
4.17	1.85**	21.64	7.18	38.78	22.82*	53.36	20.59	67.24	23.38
4.18	1.81**	21.75	7.46			53.46	22.43	67.74	22.76
4.84	1.95**	22.51	9.26*	40.50	10.96	53.71	21.93	68.57	26.42
5.29	1.71	23.96	7.70	41.20	15.17	53.75	22.15		
5.54	2.26**	25.18	10.26	41.36	15.73	53.79	16.60	70.40	22.03
5.67	1.57	24.53	6.56	41.70	17.84	53.92	14.83*	70.91	24.82
6.48	3.01	24.66	7.89	41.85	15.75	54.00	22.09	72.32	27.32
7.25	2.70	24.85	7.66	42.52	17.74	54.30	15.81*	72.34	23.82
7.29	3.26	26.23	8.74	42.83	15.81*	54.32	21.74	72.61	28.22
7.35	2.71*	26.94	8.33	43.05	18.61	54.61	16.51	73.65	23.43
7.82	3.53	27.43	10.51	43.51	16.61	54.77	25.29	75.44	27.29
8.44	2.81	27.58	12.69*	43.54	15.13	54.79	15.72	75.85	25.17
9.39	2.87	28.00	11.37	43.76	11.39*	54.94	26.42	76.52	23.00
9.89	4.26	28.57	13.61	44.23	17.25	56.41	21.03	76.66	22.32
9.90	4.88			44.53	14.72*	56.54	20.21	76.71	25.82
		30.46	13.16	44.89	15.02	56.79	21.10	79.29	22.69
10.50	5.35	30.69	10.47	45.10	17.36	56.89	14.99		
10.62	2.91	31.28	12.61	45.19	16.10	57.31	21.42	82.70	24.37
11.43	3.78	31.33	12.20*	45.22	15.67	57.39	18.24	82.79	24.59
11.62	5.61	31.49	9.81*	45.50	19.90	57.40	17.00	85.19	27.17
11.81	3.57	32.05	11.78	45.73	16.51	57.65	15.58	86.01	29.78
11.88	5.12	32.69	13.99	46.21	18.59	57.94	15.88	86.56	22.86
12.84	5.25*	33.09	11.03	47.04	11.93*	58.13	23.94		
13.28	3.83*	33.15	14.10	47.08	19.83	58.32	17.79	93.01	25.34
13.59	4.92	33.30	12.79	47.17	12.40	58.77	20.32		
13.87	4.69	33.73	13.44*	47.84	13.81	59.14	28.84		
14.78	5.85	33.77	14.71	48.00	16.25	59.22	21.59*		

TABLE 13.

Correlation between the body weight and the body length.

Ws : Body weight with sand in grams.

L : Body length in cms.

Ws \ L	3.4	4.4	5.4	6.4	7.4	8.4	9.4	10.4	11.4	12.4	13.4	14.4	15.4	16.4	f	M
3.1	8	6	4												18	4.18
6.5	2.5	0.5		4	1										8	5.44
9.9				2	3.5	0.5									6	7.15
13.3				3	2										5	6.80
16.7					4	5	1								10	8.10
20.1				1	1	2.5	0.5								5	7.90
23.5						4	1								5	9.60
26.9						2	1	1.5	0.5						5	9.50
30.3						1	1								2	8.90
33.7								5	1	2	2				10	11.50
37.1								1	1	3					5	11.80
40.5								0.5	0.5	3	2				6	12.48
43.9								1	3	3.5	0.5				8	11.96
47.3										4	2	1	1		8	13.35
50.7										1	5	2	1		9	13.73
54.1										1	5.5	3.5			10	13.65
57.5										1	4.5	2	1.5		9	13.84
60.9										1	1	1			3	13.40
64.3									1	2	1	2.5	1.5		8	13.59
67.7										1		2	1	1	5	14.60
71.1											0.5	0.5	2		3	14.90
74.5												1	1	1	3	15.40
77.9													3		3	15.40
81.3													2		2	15.40
84.7													0.5	0.5	1	15.90
88.1													1		1	15.40
91.5													1		1	15.40
f'	10.5	6.5	4.0	10.0	11.5	11.0	7.5	10.0	7.0	22.5	24.0	15.5	16.6	2.5	159	10.76
M'	3.91	3.36	3.10	10.58	13.45	20.25	23.79	33.36	42.86	47.22	51.48	59.36	71.73	73.82	37.46	

TABLE 14.

Correlation between the body weight and the body length.

W : Body weight without sand in grams.

L : Body length in cms.

W \ L	3.4	4.4	5.4	6.4	7.4	8.4	9.4	10.4	11.4	12.4	13.4	14.4	15.4	16.4	f	M
2.1	15.5	12.5	4	1											33	4.05
4.7		2.5	5.5	8.5	3	0.5									20	6.07
7.3			2	8.5	4	1.5									11	6.85
9.9				3	10	5	1								19	7.61
12.5				1	9	1									11	8.31
15.1						0.5	2.5	2.5	0.5						6	9.90
17.7						2	7	6.5	1.5		1				18	10.04
20.3						1		4	2	2		1			10	11.20
22.9								3.5	2.5	1	2				9	11.57
25.5								1	4	6	1				12	11.98
28.1							1	1	3	2.5	3.5	1	1		13	12.44
30.7							1	1	3	2.5	5.5	3			16	12.62
33.3									4.5	3	6	5	0.5		19	13.08
35.9									1	4	5.5	1	1.5		13	13.25
38.5									1	6.5	5.5	3	1		17	13.19
41.1									1	5.5	2.5	3.5	1.5		14	13.33
43.7									1	2	2.5	1.5		1	8	13.46
46.3									2				4		6	14.07
48.9											2	2	1	1	6	14.57
51.5											1	2	2		5	14.60
54.1													2		2	15.40
56.7																
59.3													2.5	0.5	3	15.57
62.9																
64.5													1		1	15.40
67.1													1		1	15.40
f'	15.5	15.0	11.5	17.0	17.0	19.5	13.5	19.5	27.0	35.0	38.0	23.0	19.0	2.5	273	10.29
M'	2.10	2.53	4.25	6.46	8.37	12.23	17.99	20.43	30.07	33.46	35.37	37.77	48.63	44.90	23.75	

TABLE 15.

Correlation between the body length and the trunk length.

L : Total body length in cms.

Ltr : Trunk length in cms.

Ltr / L	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6	10.4	f	M
3.5	17											17	2.40
4.5	1	13										14	3.14
5.5		6	5									11	3.56
6.5			11	7								18	4.31
7.5			1	13	3							17	4.89
8.5				5	12	1						18	5.44
9.5					6	5.5	0.5					12	6.03
10.5					3	13.5	4.5					21	6.41
11.5					1	9.5	14.5	3				28	6.96
12.5						2	12	21	3.5	0.5		39	7.76
13.5								17.5	13.5	3		34	8.46
14.5								3.5	8	13.5		25	9.12
15.5									3	10	5	18	9.70
16.5										0.5	1.5	2	10.20

TABLE 16.

Correlation between the body length and the tail length.

L : Total body length in cms.

Lta : Tail length in cms.

Lta / L	1.1	1.7	2.3	2.9	3.5	4.1	4.7	5.3	5.9	6.5	f	M
3.5	16.5	0.5									17	1.12
4.5	5.5	8.5									14	1.43
5.5		8.5	2.5								11	1.84
6.5		5	11	2							18	2 20
7.5			8.5	8.5							17	2.60
8.5			2	12.5	3.5						18	2.95
9.5				2.5	8	1.5					12	3.45
10.5					3.5	14	3.5				21	4.10
11.5					1.5	11.5	12.5	2.5			28	4.44
12.5					2	8.5	16	10.5	2		39	4.73
13.5						6	10.5	13.5	4		34	4.97
14.5							6	14	4.5	0.5	25	5.29
15.5								9	6.5	2.5	18	5.68
16.5									1	1	2	6.20

TABLE 17.

Correlation between the body weight and the integument.
W : Body weight without sand in grams.
I : Fresh weight of the integument in grams.

I / W	0.6	1.6	2.6	3.6	4.6	5.6	6.6	7.6	8.6	9.6	10.6	11.6	12.6	13.6	f	M
3.1	17	3													20	0.75
7.3	5	16													21	1.36
11.5		7	5	4											16	2.41
15.7			4	7	2	2	1								16	3.91
19.9			2	5	3	2	2	2							16	4.79
24.1				1	5	9	5								20	5.50
28.3					1	7	9	3	2	1					24	6.37
32.5					2	3	11	7	2						25	6.76
36.7						1	15	7	3	1					27	7.16
40.9						2	6	10	6		1				25	7.56
45.1							2	3	2	2	1				10	8.30
49.3								2	7	3	1	1			14	9.03
53.5										2	2	1			5	10.40
57.7									1	2	1	1		1	6	10.60
61.9										1					1	9.60
f	22	26	11	18	13	26	51	34	23	12	6	3		1	246	5.57
M'	4.05	11.48	14.55	15.53	23.45	27.01	33.01	36.82	42.57	48.95	50.00	53.50		57.70	27.49	

TABLE 18.

Correlation between the body weight and the sand
in the alimentary canal.

Ws : Body weight with sand in grams.
S : Sand in the alimentary canal in grams.

S \ Ws	1.45	3.45	5.45	7.45	9.45	11.45	13.45	15.45	17.45	19.45	21.45	23.45	25.45	27.45	29.45	f	M
4.56	23	4														27	1.75
10.76		8	6													14	4.31
16.96			9	6	1											16	6.45
23.16			1	7	4											12	7.95
29.36				1	1	5	4									11	11.63
35.56					1	9	6	2	1	2						21	13.55
41.76					1	3		6	5	1		1				17	15.68
47.96						2	3	6	2	6	2	1				22	16.19
54.16							1	5	4	3	9		2			24	19.28
60.36								3	5	3	2	3			1	17	19.69
66.56								1		1	1	6	2	2		13	23.30
72.76											1	2	2	3		8	25.21
78.96											1	2	1			4	23.45
85.16												2	1	1	1	5	25.85
91.36													1			1	25.45
f'	23	12	16	14	8	19	14	23	17	16	16	17	9	6	2	212	13.72
M'	4.56	8.69	15.02	20.95	27.03	36.21	37.77	49.04	50.47	50.66	57.64	67.29	71.38	72.76	72.76	38.98	

Study of *Euryale ferox* SALISB.
IV. On the Rate of Growth of the Lamina.[1]

BY

YÔNOSUKE OKADA.

(Biological Institute, Tôhoku Imperial University, Sendai.)
(With Plates XIII-XIV).

Introduction. *Euryale ferox* SALISB., one of the largest leaf-bearing plants grown in Japan proper, enjoys a most luxurious growth in some localities in the Prefecture of Toyama, viz, in Zyûnityôgata (十二町潟) and in Takaoka (高岡), where it is not a rare occurrence that as large leaves as those of *Victoria regia* are observed. On this fact I have already reported in my previous paper[2]. It is indeed a magnificent sight, in the midst of the vegetative season, to see their gigantic leaves overspreading the water surface (Pl. XIII). Not less noticeable is the rapid rate of growth with which the leaf anlage attains to the fully grown size in the course of only a few days. It is generally recognized that the growth of aqueous plants is executed with much rapidity as a general rule,[3] yet the case of *Euryale* may be accepted to be one of the most remarkable examples, so that it may be worth while, from the biological point of view, to study the actual rate of growth of the lamina. The following paragraphs are mainly the results of my study at Zyûnityôgata last summer.

On the season of the vigorous growth. So far as the observations

up to this day are concerned, the largest leaves of *Euryale* appear from the end of August to as late as the middle of September. It may therefore be presumed that this period is the most favorable time for the vegetation. The real cause of this relationship must be highly complicated, and a comprehensive analysis cannot be made at all with mere observations. But taking into consideration that the temperature ' is the most effective of all the environmental factors influencing the growth rate of plants, it is not irrational to expect to find some relationships here between the temperature and the vegetative vigour of *Euryale*.

The body of the plant, in the main, is exposed partly to the water and partly to the atmosphere, so that we must take into account both of these surrounding media in considering the temperature relation. Of these two, for the atmospheric temperature, we can resort to the record of the Husiki Meteorological Observatory (some 9 km. from Zyûnityôgata), which tells that the weekly average attains the maximal value usually in the second or the third week of August (Table 1).

TABLE 1.   Weekly average of atm. temp. for August.
(Husiki Meteorological Observatory)

	1924	1925	1926	1927	1928
1st. week	28.16	25.46	25.99	25.47	26.12
2nd. „	27.67	26.21	26.03	25.69	25.46
3rd. „	27.70	26.57	25.47	25.72	26.57
4th. „	24.84	26.05	25.83	25.60	24.46

As for the temperature of the water at the bottom (some 50 cm. deep), I have been favored by Mr. MATOBA's kind effort, a semimonthly record of one round year, according to which we know that the maximum (32.5°C) takes place at about the beginning of September. (The complete record will be given in my later paper in connection with the study of the habitat.)   From these two records it is suggested that the water temperature lags to some degree after the atmospheric temperature, and the plant body which is situated between these two

exhibits its full vigour of vegetative activity in the period between these two maxima.

On the rate of the increase in the surface of the leaf blade. According to the above reasoning, the latter half of August towards the beginning of September seems to be most fitted to study the growth of the lamina of *Euryale,* so that I made a visit to the *Euryale-* vegetation at Zyûnityôgata at the end of August, and accomplished some measurements concerning the growth rate of the leaf blade. As a prolonged stay there was not afforded, and accordingly continuous study with one and the same material throughout the developmental course was not to be aimed at, I was compelled to resort to measurement with random samples. For two days during my stay there, diameters of the leaf blades were measured twice daily at the 8th and 17th hours, and from these values, the areas of the surface (or to be more exact, the areas of the projection of the leaf blade on the water surface) were computed.

The general outline of the lamina may be called roughly circular if we ignore such minor irregularities as the process at the leaf tip or the small insinuation at the base (Pl. XIV). The diameters are, however, not perfectly equal in all directions, the one along the midrib (Textfig. 1, AB) being a little shorter than the one across (Textfig.
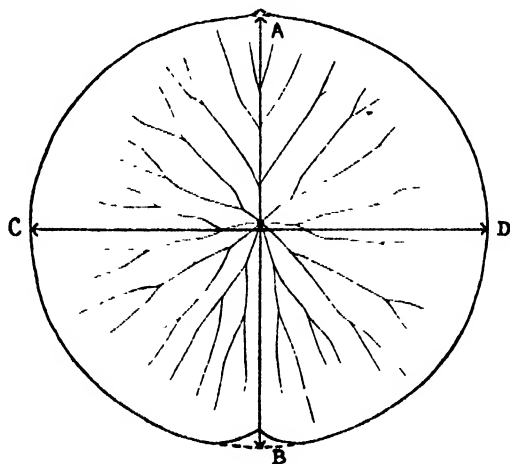
Textfig. 1. Outline of the lamina.

TABLE 2.   Diameter and area of the leaf blade.

Date and hour		24/VIII, 8h.	24/VIII, 17h.	25/VIII, 8h.	25/VIII, 17h.	26/VIII, 8h.
Temperature	Air	23.0°	22.7°	23.0°	26.0°	24.5°
	Water surface	28.0°	28.7°	26.0°	29.0°	27.3°
	Water bottom (50 cm.)	28.0°	29.0°	28.0°	28.6°	28.0°
Diameter in cm.	a.	51	56	64	69	73
	b.	52	55	58	60	63
	c.	67	73	81	85	89
	d.	78	80	82	83	85
	e.	129	130	131	132	132
	f.	136	138	139	140	141
	g.	139	141	142	143	144
	h.	157	158	158	159	159
Area in sq. dm.	a.	20.43	24.63	32.18	37.39	41.85
	b.	21.24	23.76	26.41	28.28	31.17
	c.	35.26	41.85	51.53	56.74	62.22
	d.	47.78	50.27	52.80	54.12	56.74
	e.	130.7	132.7	135.1	136.9	136.9
	f.	145.2	149.6	151.7	153.9	156.2
	g.	151.7	156.2	158.4	160.6	162.9
	h.	193.6	196.1	196.1	198.6	198.6

TABLE 3.   Increment of area per hour (in sq. cm.)

	24/VIII, 8h. — 24/VIII, 17h.	25/VIII, 8h. — 25/VIII, 17h.	26/VIII, 8h.
a.	47	50	58	30
b.	28	18	21	20
c.	73	65	58	37
d.	28	17	15	18
e.	20	16	20	0
f.	50	14	24	15
g.	50	15	24	15
h.	30	0	26	0

1, CD). In our record the arithmetical mean of the above two values is taken for the diameter and the area is computed approximately as a circle.

The result of the measurement is arranged in Tables 2 and 3.

From these results we know the fact that the act of growth takes place both in the daytime and in the night, and further that the growth rate in the former seems as a general rule slightly more favored than in the latter. As for the question as to the growth rate in relation to the age, we cannot know much, owing to the

TABLE 4. Growth rate of the lamina of *Euryale* at Takaoka observed by Mr. OTAYA.

No. 1.

Date	Aug. 26	27	28	29	30	31	Sept. 1	2
Length of the lamina (cm.)	81.5	100.0	115.0	127.5	130.5	138.0	143.0	143.0
Length of CD* (cm.)	85.5	108.7	125.0	137.0	142.5	150.0	154.5	154.5
Length of AB* (cm.)	82.0	103.0	120.0	134.0	140.0	143.0	151.0	151.0
Area (sq. cm.)	5836	8817	11869	14516	15606	16956	18375	18375
Daily increment of CD (cm.)		23.7	16.3	12.0	5.5	7.5	4.5	0
Daily increment of area (sq. cm.)		3281	3052	2647	1090	1350	1419	0
Remarks	Sunny	Sunny	Sunny	Sunny	Cloudy, with light rain	Sunny strong wind	Sunny	Sunny
Water temperature		32°	32°	32.5°	29°	33°	31.5°	31°

The measurement was commenced on the third day of the horizontal expansion of the lamina, and the completion was attained on the seventh day of measurement, so that the whole period of the horizontal expansion of the lamina is reckoned to be about nine days. The same holds good in the next example as well.

*)Those symbols refer to Textfig. 1.

No. 2.

Date	Aug. 26	27	28	29	30	31	Sept. 1	2
Length of the lamina (cm.)	77	103.8	113.5	123.0	133.5	136.0	140.5	140.5
Length of CD (cm.)	86	111.0	128.5	139.5	148.0	150.0	153.5	153.5
Length of AB (cm.)	80	108.0	124.0	133.4	146.0	147.0	151.5	151.5
Area (sq. cm.)	5401	9326	12450	14627	16962	17427	18061	18061
Daily increment of CD (cm.)		25	17.5	11	8.5	2.0	3.5	0
Daily increment of area (sq. cm.)		3925	3124	2177	2335	465	634	0

limited period of observation, beyond the vague idea that the increment falls with the age of the leaf in most cases.

This serious gap in my study was later filled by Mr. OTAYA, who continuously performed every twenty-four hour's measurement with some specified leaves of *Euryale* at Takaoka. Examples from his protocols are tabulated below with his permission.

His result shows the general tendency that, apart from the extremely young stage in the development, the daily increment falls considerably with age, not only in the diameter but also in the area. Another remarkable point is the rapidity in growth expressed in the actual increase in the diameter. The largest value observed attains as much as 25 cm. per day, that is, the leaf of *Euryale* expands more than 1 cm. across per hour in its prime of vegetative vigour.

The mass of the leaf as related to its surface. The surface of the lamina is not simply plane but is coarsely wavy, and the irregularity is, in particular remarkable in the younger leaves that they may be described as coarsely rugous (Plate XIV). In the tables above, these irregularities are neglected and the area is computed from the length of the diameter under the assumption that the outline is perfectly circular, so that the area in these tables denotes that of the projection, so to speak, of the lamina on the water surface, but not the true
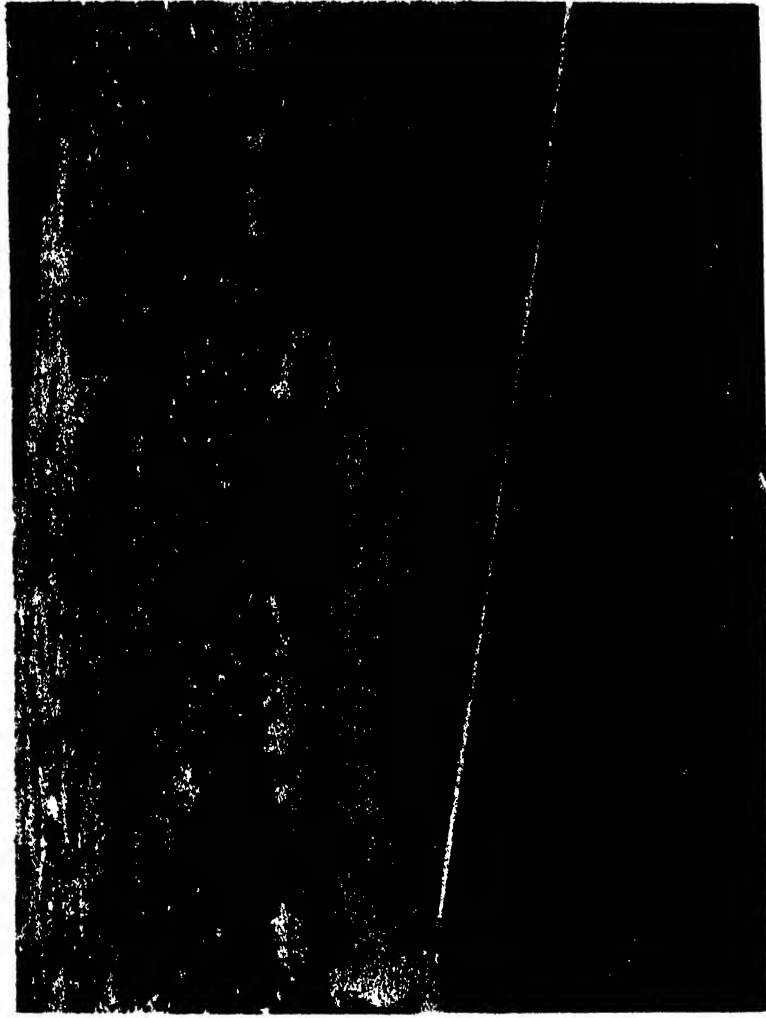
Y. OKADA : *Euryale ferox*, SALIS-B.

surface area of the lamina. Now, it is obviously more rational to measure the actual leaf surface than its projection in studying the growth phenomenon of the leaf. The practice is, however, almost impossible. An alternative is to denote the increment in terms of the mass of the leaf instead of the area. But, here, too, is the ungovernable difficulty of weighing, undetached from the mother plant, the leaf blade which still keeps growing on in the water, and I was compelled to resort to the indirect and imperfect method of computing the ratio of the mass to the area of the leaf in various stages of development and then interpreting the change in the area into that in the mass.

The result of my calculation of the above ratio with random samples is shown in Table 5 and in Textfig. 2.

We know therefrom that the smaller the diameter is, the ratio of the mass to the area is the larger.

TABLE 5.   The mass of the lamina in
relation to the area.

	Diameter cm.	Area sq. cm.	Fresh weight kg.	Dry matter		Dry matter/Area mg/sq. cm.
				g.	%	
a.	32	804.1	0.6	25.0	4.17	31.09
b.	47	1734	0.86	39.8	4.63	22.95
c.	74.1	4359	2.01	74 4	3.70	17.07
d.	97.5	7461	3.5	154.8	4.42	20.75
e.	116	10560	3.4	148.2	4.36	14.04
f.	126	12460	3.6	154.2	4.28	12.38

As is previously stated, the rate of increase in the area of the lamina falls with age. Now we know furthermore that the ratio mass/area decreases too with age. Taking these two tendencies in combination, the result is that the increment of the mass of the lamina suffers from considerable decline as the age goes on and the diameter grows.

The dry matter in percent of the fresh weight. In the sixth column of the above table is attached the value of the dry matter content
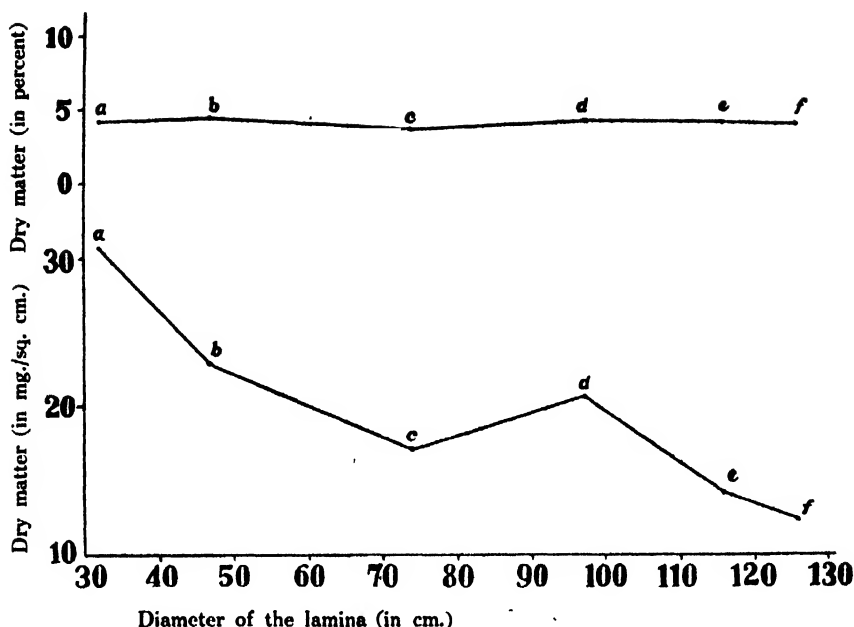
Textfig. 2. The ratio mass/area and dry matter content as related to the size of the lamina.

of the lamina expressed in percent of the fresh weight, which shows that it is almost constant for any sample and therefore independent of the size or age of the leaf itself. The remarkably low value of the percentage may be accepted as a characteristic common to aqueous plants in general.

In conclusion, I wish to acknowledge my indebtedness to Mr. MATOBA and Mr. OTAYA for their kind help during my study, and especially to the former for his kindness in recording the water temperature and to the latter for his liberal permission to refer to his unpublished data.

EXPLANATION OF THE PLATES.

Pl. XIII. *Euryale ferox* SALISB. in its natural habitat at Zyûnityôgatâ, showing a gigantic leaf blade measuring 191 cm. (6.3 Syaku) across.

Pl. XIV. The same with its under surface exposed. Attached is an unexpanded young leaf showing the conspicuous rugous markings on the upper surface.

Über die Chromosomenzahlen bei einigen Potentillen.

VON

Naomasa Shimotomai.

(Biologisches Institut der Kaiserlichen Tôhoku Universität, Sendai.)

Potentilla, eine Gattung von *Rosaceae*, ist sehr artenreich. Nach Wolf (1908) umfasst sie etwas über 300 Arten, von denen die meisten von der nördlichen gemässigten Zone bis in die arktische hinein weit verbreitet sind und einige in der südlichen gemässigten Zone vorkommen.

Diese grosse Gattung ist aber hinsichtlich, der Chromosomenforschung bis vor kurzem fast ganz vernachlässigt worden, indem nur wenige Arten von einigen Forschern untersucht wurden. 1908 untersuchte Tischler einen sterilen Artbastard zwischen *Potentilla Tabernaemontani* und *P. rubens* (=*opaca*) zytologisch und fand 32 Chromosomen bei der somatischen und 16 bei der meiotischen Teilung. Ganz dieselbe haploide Zahl fand er auch bei dessen Eltern. 1914 nahm Forenbacher die Chromosomenzählung bei 4 Arten vor, von denen nach ihm eine Art 8 und drei andere 16 haploide Chromosomen haben. 1927 untersuchte Frl. Roscoe eine anscheinend triploide Riesenform von *P. anserina*, aber sie konnte keine genaue Chromosomenzahl bei dieser Form feststellen. Die Angaben der beiden ersten Autoren scheinen, uns zu zeigen, dass Chromosomenpolyploidie mit der Grundzahl 8 bei dieser Gattung auftritt.

Ganz kürzlich hat Tischler (1928) abermals zwei Potentillen untersucht. Als haploide Chromosomenzahl fand er aber bei *Potentilla alba* 14 und bei *P. aurea* am wahrscheinlichsten 28. Diese neue Entdeckung brachte den Autor auf den Gedanken, dass die Grundzahl der Chromosomen bei dieser Gattung wie bei den naheverwandten Gattungen, z. B. *Rubus* u. a., auch 7 sein müsse und dass die früheren Angaben, die die Zahlen 8 und 16 berichteten, ganz irrig wären.

Auch in Japan gibt es etwa 30 Arten dieser Gattung. Um einerseits diese einheimischen Potentillen zytologisch näher zu erkennen

und anderseits die oben erwähnte, von TISCHLER vorgeschlagene Frage
zu beantworten, habe ich eine zytologische Untersuchung über diese
Gattung untergenommen. In vorliegender kleiner Arbeit teile ich die
Chromosomenzahlen nur einiger Arten mit. Über die Chromosomen-
zahlen der anderen zahlreichen Arten wird in den nachfolgenden Mit-
teilungen berichtet werden.

Zuerst untersuchte ich im Sommer dieses Jahres die meiotische
Teilung bei *Potentilla chinensis*, da diese Pflanze gerade in ihrer
Blütezeit war. Die Pollenmutterzellen wurden mit BELLINGscher
Eisenessigkarminlösung fixiert
und gefärbt. Damit lassen sich
die metaphasichen, bivalenten
Chromosomen ziemlich gut fär-
ben. Sie haben die Gestalt
eines kleinen, kurzen, dicken
Stäbchens. Fig. 1 zeigt die
Polansicht einer Kernplatte bei
heterotypischer Metaphase, wo
man am deutlichsten 7 Chro-
mosomen wahrnehmen kann.
Diese Beobachtung interessiert
uns deshalb, weil wir hier bei

Fig. 1. Eine Polansicht heterotypis-
cher Metaphase in P. M. Z. von *Potentilla
chinensis*. (×2100).

unserer Art die von TISCHLER schon vermutete, aber noch nicht
wirklich erwiesene Grundzahl bei dieser Gattung gefunden haben.[1]

Um noch andere einheimische Potentillen zu untersuchen, habe
ich in diesem Herbst zur Zählung der somatischen Chromosomen
diesmal die Wurzelspitzen fixiert, welche man von in Töpfen gepflanzten
Pflanzen leicht nehmen konnte. Die Fixierung geschah mit FLEMMINGS
Gemisch nach dem Rezept des Bonn-Instituts. Paraffinschnitte wurden
mit HEIDENHAINS Eisenalaunhämatoxylinlösung gefärbt.

Die somatischen Chromosomen sind stäbchen- oder hakenförmig
und sehr klein, können jedoch ohne Schwierigkeit gezählt werden,
da sie gewöhnlich nicht aneinander kleben (Fig. 2). Die Chromsomen-
zahlen der hier untersuchten Arten seien samt derselben der vorhin
beschriebenen im Folgenden angegeben:

---

[1] Neulich hat mir Herr Prof. Dr. TISCHLER brieflich mitgeteilt, dass auch er bei
Potentilla verna u. a. 7 haploide Chromosomen beobachtet hat.

Fig. 2.   Somatische Kernplatten bei Potentillen. (×3200)
1, *P. Kleiniana*; 2, *P. fragarioides*; 3, *P. Matsumurae*; 4, *P. nipponica*.

		n	2n
Potentilla chinensis, SER.		7	
P.	*fragarioides*, L.		14
P.	*Kleiniana*, WIGHT et ARN.		14
P.	*Matsumurae*, WOLF.		28
P.	*nipponica*, WOLF.		28

Wie die Tabelle zeigt, sind die drei ersteren Arten diploid und die beiden letzteren tetraploid.

Durch TISCHLERS und meine eigene Arbeiten kennen wir jetzt bei dieser Gattung die reduzierten Zahlen 7, 14 und ca. 28.   Diese Zahlen scheinen eine Serie regelrechter Polyploidzahlen zu bilden.

Unter Potentilloideen weisen zwei Gattungen, *Rubus* (LONGLEY 1924) und *Fragaria* (ICHIJIMA 1926), schöne Chromosomenpolyploidien mit der Grundzahl 7 auf.   Ganz dieselbe Polyploidie können wir auch bei der Gattung *Geum* erwarten, denn WINGE (1924) stellte bei zwei Arten von *Geum* 21 haploide Chromosomen fest.   Also liegt es nahe, anzunehmen, dass bei sämtlichen Potentilloideen ganz dieselbe Chromosomenpolyploidien mit der Grundzahl 7 vorkommen.

Zum Schluss möchte ich Herrn Prof. Dr. TAHARA für seine liebenswürdige Unterstützung meinen besten Dank aussprechen.

Dezember 1928.

LITERATURVERZEICHNIS

FORENBACHER, A. 1914. Die Fortpflanzungsverhältnisse bei der Gattung *Potentilla*. Acad. d. scienc. et des arts des Slaves du sud de Zagreb, S. 86–97.

ICHIJIMA, K. 1926. Cytological and genetic studies on *Fragaria*. Genetics, Vol. 11, pp. 590–604.

LONGLEY, A. E. 1924. Cytological studies in the genus *Rubus*. Amer. Journ. of Bot., Vol. 11, pp. 249–282.

ROSCOE, M. V. 1927. Meiotic irregularities in a gigas form of *Potentilla anserina*. Bot. Gaz., Vol. 84, pp. 307–316.

TISCHLER, G. 1908. Zellstudien an sterilen Bastardpflanzen. Archiv f. Zellforsch., Bd. 1, S. 33–151.

TISCHLER, G. 1928. Über die Verwendung der Chromosomenzahlen für phylogenetische Probleme bei den Angiospermen. Biolog. Zentralbl., Bd. 48, S. 321–345.

WINGE, Ö. 1924. Contributions to the knowledge of chromosome numbers in plants. La Cellule. Tom. 35, p. 305–324.

WOLF, TH. 1908. Monographie der Gattung *Potentilla*. Bibliotheca Botanica, Bd. 16, Heft 71.

NACHSCHRIFT. Nach der Vollendung dieser Mitteilung empfing ich die ganz kürzlich erschienene Arbeit von ARNE MÜNTZING, „Pseudogamie in der Gattung *Potentilla* (Hereditas, Bd. 11, S. 267–283, 1928)". Auch er fand, dass die Grundzahl der Chromosomen bei dieser Gattung 7 ist.

Über eine tetraploide Gartenrasse von *Psilotum nudum*, Palisot de Beauvois (= *P. triquetrum*, Sw.) und die tripolige Kernteilung in ihren Sporenmutterzellen.

Von

Sakuichi Okabe.

(Biologisches Institut, Kaiserl. Tôhoku Universität, Sendai.)

(Mit Tafel XV.)

Seit langem wurde *Psilotum nudum*, Palisot de Beauvois (= *P. triquetrum*, Sw.) von verschiedenen Forschern zytologisch studiert. Bekanntlich wurden die Sporenmutterzellen dieser Pflanze wegen ihres grossen chromatinreichen Kerns um das Ende des vorigen Jahrhunderts von europäischen Zytologen als günstiges Material für Untersuchungen der Kernteilungsvorgänge, Polkörperchen und Zellteilungen gebraucht. Weit später, vor neun Jahren (1920), hat Yamaha die Zellteilung, besonders die Zellplattenbildung der Archesporen und Sporenmutterzellen genau untersucht.

Psilotum nudum, das in Südjapan wild vorkommt, wurde einst von japanischen Gärtnern als Zierpflanze viel gezogen. Wenigstens über 100 Gartenrassen bekamen ganz phantastische Namen. Aber jetzt werden leider nur noch wenige von ihnen gelegentlich in Gewächshäusern botanischer Gärten oder in privaten Gärten gefunden.

Als ich im vorigen Herbst einige Präparate der Sporenmutterzellen einiger Rassen dieser Pflanze, die im hiesigen Gewächshause gezüchtet werden, zwecks Bestimmung der Chromosomenzahlen prüfte, habe ich zu meinem Erstaunen etwas Sonderbares, hochchromosomige und tripolige Kernteilung, beobachtet. Meine Beobachtungen teile ich hier kurz mit.

Als Fixierungsmittel benutzte ich meistens Flemmings Chromosmiumessigsäurelösung in Bonner Konzentration; Bouins Lösung war auch brauchbar. Die Färbung der 15~25 μ dick geschnittenen Paraffinschnitte geschah mit Heidenhains Eisenalaunhämatoxylin.
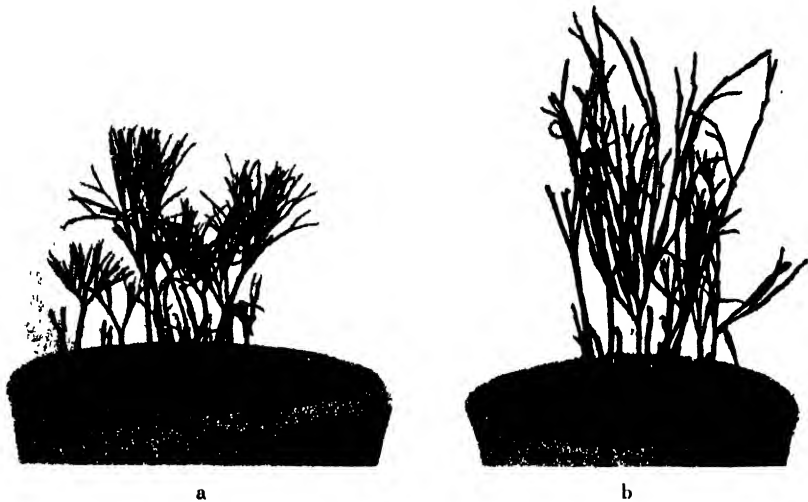
Textfig. 1. Zwei Gartenrassen von *Psilotum nudum*, PALISOT DE BEAUVOIS a, Eine diploide Rasse (Hakuryū). b, Eine tetraploide Rasse. Gartenname unbekannt. Die Sporangien beider Pflanzen wurden für meine Untersuchungen weggenommen. Die tetraploide Rasse ist viel grösser als die diploide. ca. ¼

Zuerst über die Chromosomenzahlen. Wenn man das klassische Bild HOFMEISTERS (1867) sicht, ist es leicht, 55 Gemini in seiner Fig. 16, e zu unterscheiden. STRASBURGER (1884) hat dagegen eine noch höhere Zahl, ca. 140, gezählt. 1896 richtete ROSEN seine besondere Aufmerksamkeit auf die Chromosomenzahlen dieser Pflanze, und er schrieb, „Die Zählung ergiebt hier 50 Chromosomen, und da doppelte Zähluug bei der Krümmung eines Segmentes aber kaum vorkommen konnte, so wird man die Zahl auf 48 reduciren dürfen."

Bei meinem Material besitzt eine Rasse in der ersten meiotischen Teilung 52 (Textfig. 2. a) und die andere 104 Gemini (Textfig. 2. b). Letztere ist also als tetraploide Rasse anzusehen. Ich vermute, dass das von STRASBURGER benutzte Material auch eine tetraploide Rasse war.

Wie schon ROSEN bemerkte, ist es etwas schwer, die auf der Kernplatte angeordneten Chromosomen richtig zu zählen, weil sie verschieden geformt und nebeneinander zusammengedrängt sind. Zur Zählung der reduzierten Chromosomenzahlen ist das Diakinesestadium noch zweckdienlicher als die Metaphase, denn hier sind die Gemini

längs der Kernperipherie zerstreut angeordnet. In diesem Stadium konnte ich durch Änderung der Einstellung 52 resp. 104 Gemini und je einen Nukleolus konstatieren.

Die Verlauf der Reduktionsteilung der diploiden Rasse geht ganz regelmässig vor sich, und viele normale Tetraden werden daraus gebildet. Im Gegensatz dazu treten in den tetraploiden Sporenmutterzellen viel weniger normale Teilung als tripolige auf.
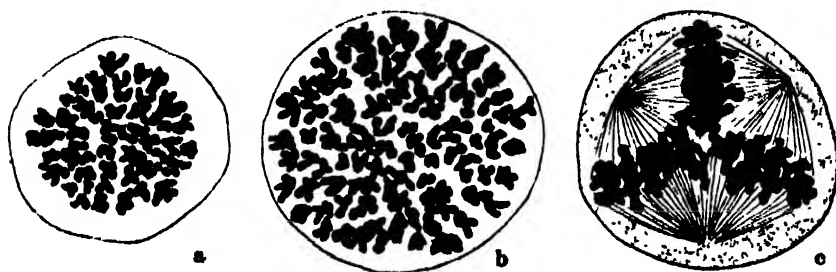
Textfig. 2. Metaphasische Kernplatten der ersten meiotischen Teilung der Sporenmutterzellen von *P. nudum* (a, diploide Rasse, b, und c, tetraploide Rasse). × 1000.

a, Polansicht der 52 chromosomigen Platte. b, Polansicht der 104 chromosomigen Platte. c, Seitenansicht der dreiteiligen Platte.

Es ist schon bekannt, dass sich unter nahe verwandten Rassen oder Arten das Volumen der Kerne gemäss der Erhöhung der Chromosomenzahlen vergrössert. Die unten wiedergegebene Tabelle zeigt dieses Verhältnis auch ganz klar. Die Messung ist in der Diakinese ausgeführt worden.

	Chromosomen-zahl	Kerndurch-messer	r³	Zahl der gebrauchten Zellen
Diploide Rasse	52	19.73 μ ± 1.95 μ	296.06 μ	227
Tetraploide Rasse	104	25.62 μ ± 2.00 μ	648.60 μ	223

Dass die tripoligen Teilungen, zusammen mit anderen Abnormitäten, durch experimentelle Behandlung hervorgerufen werden, ist von verschiedenen Autoren schon wiederholt berichtet worden. Ich werde

hier nur einige Fälle, wo die tripolige Teilung bei meiotischen Teilungen auftritt, anführen. Durch Chloralisierung hat SAKAMURA (1920) tripolige Teilung in der heterotypen Metaphase der Pollenmutterzellen von *Vicia faba* gefunden. Solche Fälle sind ferner von MICHAELIS (1926) in durch Kälte beeinflussten Pollenmutterzellen von *Epilobium* bei hetero- und homöotypen Teilung und von YAMAHA (1927) in mit Äthyläther behandelten heterotypen Metaphasen von *Daphne odora* beobachtet worden. FUKUDAS Untersuchungen nach (1927) treten diese Teilungen bei einigen Rassen der Kartoffelpflanze bei abnormen hetero- und homöotypen Teilungen, die aber bei niedriger Temperatur normal sind,[1] häufig ein. HEITZ (1925) hat auch tripolige Teilungen in den Pollenmutterzellen von *Melandrium album* gefunden und meint, dass auf diese Weise später auch Zwitter aus dieser Pflanze hervorgehen würden.

In den ersten Metaphasen der Sporenmutterzellen der tetraploiden Rasse von *P. nudum* treten sehr deutlich tripolige Spindeln auf (Textfig. 2. c). Obwohl schon von vielen Forschern verschiedene Abweichungen in den Kernteilungsvorgängen aufgewiesen wurden, scheint es mir doch der Mühe wert, das quantitative Verhältnis einer Abnormität zu berücksichtigen. Ich hatte bei dieser tetraploiden Pflanze gute Gelegenheit, das Zahlenverhältnis beim Auftreten der tripoligen Teilung zu beobachten. Wie die unten beigefügte Tabelle zeigt, ist es sehr auffallend, dass es da beinahe 80% tripolige Teilung gibt.

Nr. der Präparate / Teilungsmodus	I.	II.	III.	Summe	%
Bipolige Teilung	50	20	44	114	20.14
Tripolige Teilung	141	94	217	452	79.86

Zunächst will ich mit einigen photographischen Bildern die Vorgänge der tripoligen Kernteilung erläutern. Die Prophase verläuft regelmässig; danach ordnen sich die Gemini in drei Teile. Aus Pl.

---

[1] STOW, I. 1927. A cytological study on pollen sterility in *Solanum tuberosum* L. Jap. Journ. of Bot. Vol. 3, pp. 217–238.

XV, Fig. 7, Textfig. 2. c und Textfig. 3. a lässt sich der Ordnungsstand der Gemini leicht erkennen. Die zwei ersteren vergegenwärtigen die Seitenansichten der ersten Metaphase, wo die Gemini ⋏ förmig gesammelt und deutlich tripolige Spindeln gebildet sind. Diese Bilder können als Sammlung von drei Teilungsfiguren, deren Spindeln in drei Polen zusammengekommen sind, betrachtet werden. In Textfig. 3. a werden ausser zwei solchen Seitenansichten vier andere Ansichten der dreiteiligen Kernplatten gezeigt, wo die Pol- und etwas schräge Seitenansicht der halbmondförmigen Plättchen nebeneinander liegen. Im Präparate wird durch Änderung der Einstellung das dritte Plättchen ohne Schwierigkeit erkannt. Mit Fortschreiten des Teilungsprozesses trennen sich die anaphasischen Chromosomen voneinander und wandern allmählich nach den drei Polen (Pl. XV, Fig. 8 und 9) ab. Infolgedessen haben die Interkinesezellen drei Kerne, dann kommen drei homöotype Kernplatten in einer Zelle vor (Pl. XV, Fig. 10 und 11).

a   b
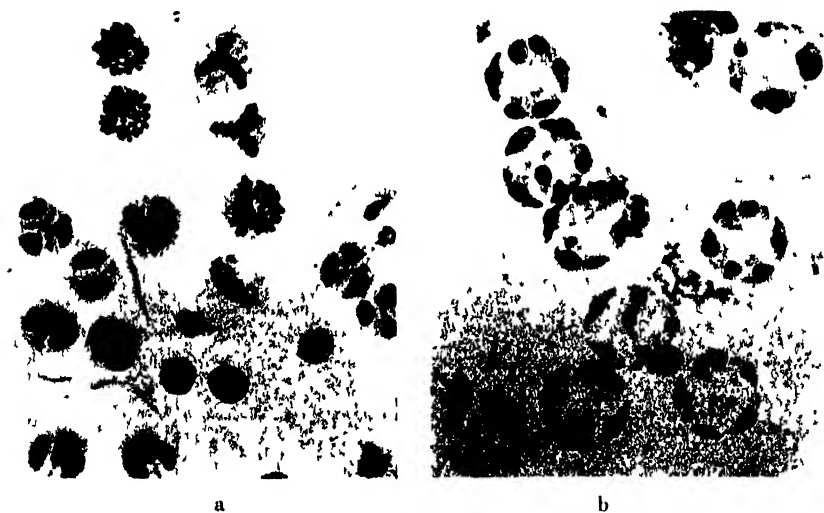
Textfig. 3. a, Ein Teil des Sporangiums. Sechs dreiteilige Kernplatten und viele dreikernige Interkinesezellen sind zu sehen × 250. b, Sporenhexaden, die durchs Deckglas etwas verdeckt sind (Essigkarminpraparat) ca × 150.

Wie nach Obenerwähntem zu erwarten ist, erscheinen nach Vollendung der homöotypen Teilung viele Hexaden (Textfig. 3. b). Wenn die zur Zählung verwendeten Zellen auch wenig sind, so genügt wohl

die folgende Tabelle doch, das Zahlenverhältnis im Vorkommen der Tetraden, Pentaden, Hexaden, u. s. w. zu zeigen.

Zahlen der in einer Zelle enthaltene Kerne	4	5	6	7	8	9	10
Zahlen der Zellen	35	46	75	17	5	2	1

Die Zählung erfolgte beim Essigkarminpräparat. Es erscheint etwas verwunderlich, dass fünfkernige Zellen ziemlich häufig vorkommen. Das dürfte auf der normalen homöotypen Teilung durch Zerstreuung der Chromosomen sowie auf der Verschmelzung der zwei Kerne bei dieser tripoligen Teilung beruhen.

Die reifen Sporen dieser tetraploiden Rasse scheinen meistens so gesund wie die der diploiden zu sein. Wenn aus diesen Sporen fertile Nachkommen gezüchtet werden könnten, so würden sie geeignetes Material zur Klärung verschiedener Fragen der Artbildung liefern.

Zum Schluss sei es mir gestattet, meinem hochverehrten Lehrer, Herrn Prof. Dr. M. TAHARA, für seine freundliche Unterstützung meinen herzlichen Dank auszusprechen.

den 10. März 1929.

LITERATURVERZEICHNIS.

1) FUKUDA, Y. 1927. Cytological studies on the development of the pollen-grain in different races of Solanum tuberosum L., with special reference to sterility. Bot. Mag. Tokyo, Vol. 41, pp 459–476.

2) HEITZ, E. 1925. Unregelmässigkeiten bei der Reduktionsteilung von Melandrium album Ber. d. D. Bot. Ges., Bd. 43, S. 77–80.

3) HEITZ, E. 1926. Beitrag zur Cytologie von Melandrium. Planta, Bd 1, S. 241 259.

4) HOFMEISTER, W. 1867. Die Lehre von der Pflanzenzelle Leipzig

5) HUMPHREY, J. E. 1894. Nucleolen und Centrosomen. Ber. d. D. Bot. Ges., Bd. 12, S. 108–117.

6) KARSTEN, G. 1893. Über Beziehungen der Nucleolen zu den Centrosomen bei Psilotum triquetrum. Ber. d. D. Bot. Ges., Bd. 11, S. 555 562.

7) KÖRNICKE. M. 1905. Über die Wirkung von Röntgen- und Radiumstrahlen auf pflanzliche Gewebe und Zellen. Ber d D. Bot. Ges., Bd. 23, S. 404–415.

8) KOMURO, H. 1924. The cells of Vicia faba modified by Röntgen rays, and their resemblace to malignant tumour cells with the cytological observations of tumours. Jap. Journ. Bot., Vol 2, pp. 133–156.

9) MICHAELIS. P, 1926. Über den Einfluss der Kälte auf die Reduktionsteilung von *Epilobium*. Planta, Bd. 1, S. 569–582.

10) MOLISCH, H. 1927. Im Lande der aufgehenden Sonne. Wien.

11) NĚMEC, B. 1910. Das Problem der Befruchtungsvorgänge und andere cytologische Fragen. Berlin.

12) ROSEN, F. 1896. Beiträge zur Kenntniss der Pflanzenzellen, 3. Kerne u. Kernkörperchen in meristematischen u. sporogenen Geweben. Cohns Beitr. z. Biol. d. Pfl., Bd. 7, S. 225–313.

13) SAKAMURA, T. 1920. Experimentelle Studien über Zell- und Kernteilung mit besonderer Rücksicht auf Form, Grösse und Zahl der Chromosomen. Journ. of the Coll. of Sci., Imp. Univ. of Tokyo, Vol. 39, Art. 11, S. 1–221.

14) STEIN, E. 1927. Untersuchungen über die Radiomorphosen von *Antirrhinum*. Zeitschr. f. indukt. Abst. u. Vererb. -Lehre, Bd. 43, S. 1–87.

15) STRASBURGER, E. 1880. Zellbildung und Zellteilung. 3te Aufl. Jena.

16) STRASBURGER, E. 1884. Die Controversen der indirecten Kernteilung. Arch. f. mikr. Anat., Bd. 33, S. 246–304.

17) TAKAMINE, N. 1923. On the effect of ultraviolet rays upon nuclear division of plants. Bot. Mag. Tokyo, Vol. 37, pp. 109–112.

18) TISCHLER, G. 1921-1922. Allgemeine Pflanzenkaryologie. Berlin.

19) TISCHLER. G. 1928. Über die cytologischen Phänomene bei der Gonensterilität der Angiospermen. Österr. Bot. Zeitschr., Bd. 77, S. 292–306.

20) YAMAHA, G. 1920. Einige Beobachtungen über die Zellteilung in den Archesporen und Sporenmutterzellen von *Psilotum triquetrum* Sw., mit besonderer Rücksicht auf die Zellplattenbildung. Bot. Mag. Tokyo, Vol. 34, S. 117–129.

21) YAMAHA, G. 1927. Experimentelle zytologische Beiträge. I Mitteilung. Orientierungsversuche an den Wurzelspitzen einiger Pflanzen. Journ. of the Faculty of Sci., Imp. Univ. Tokyo, Sect. III, Botany, Vol. 2, Part. 1, S. 1–214.

22) YAMAHA, G. 1927. Experimentelle zytologische Beiträge. III Mitteilung. Über die Wirkung einiger Chemikalien auf die Pollenmutterzellen von *Daphne odora* THUNB. Bot. Mag. Tokyo, Vol. 41, S. 181–211.

ERKLÄRUNG DER TAFEL XV.

Sämtliche photographische Abbildungen wurden mit Hilfe eines Leitzschen mikrophotographischen Apparats ausgeführt, unter Benutzung des Leitzschen Achromat-Objektivs 7 und des Leitzschen Periplanokulars ×10. Vergrösserung 1000.

Fig. 1-3. Sporenmutterzellen der diploiden Rasse von *Psilotum nudum.* (Hakuryû)

Fig. 1. Diakinese.
Fig. 2. Heterotype Metaphase in Polansicht.
Fig. 3. Dasselbe Stadium in Seitenansicht. Bipolige Spindel ist klar zu sehen.

Fig. 4-12. Sporenmutterzellen der tetraploide Rasse von *Psilotum nudum.*

Fig. 4. Diakinese.
Fig. 5. Normale heterotype Kernplatte in Polansicht.
Fig. 6. Dieselbe in Seitenansicht. Bipolige Spindel ist sichtbar.
Fig. 7. Dreiteilige heterotype Kernplatte mit tripoliger Spindel in Seitenansicht.
Fig. 8. Frühere Anaphase der tripoligen Teilung.
Fig. 9. Spätere Anaphase der tripoligen Teilung.
Fig. 10. Interkinesezelle mit drei Kernen. Körnige Scheidewand ist bemerkbar.
Fig. 11. Homöotype Metaphase mit drei Kernplatten. Eine Polansicht und zwei Seitenansichten sind zu sehen.
Fig. 12. Homöotype Anaphase.
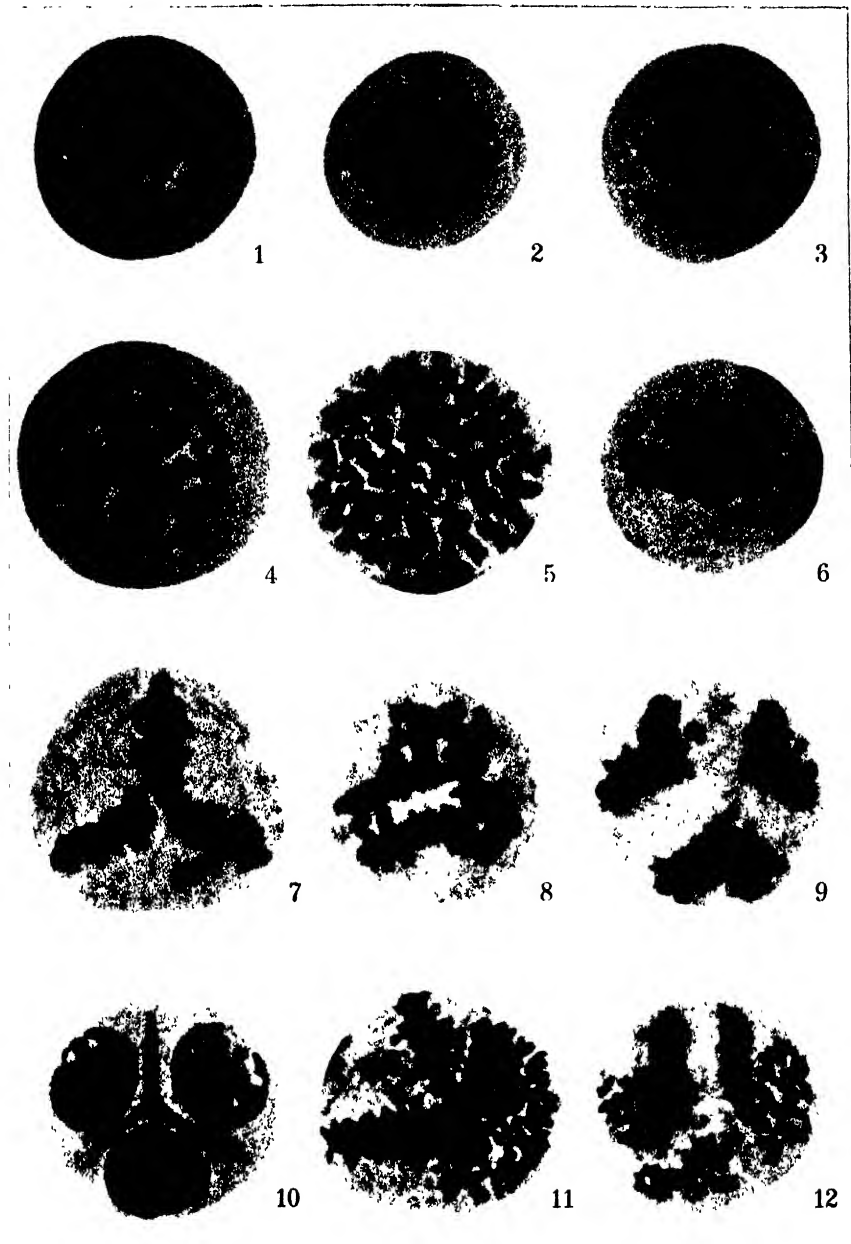
S. Okabe: Tetraploide Gartenrasse von *Psilotum nudum*.

Embryologie der *Liliaceae*, mit besonderer Rücksicht auf die Endospermbildung.
I. *Melanthioideae* und *Aletroideae*.[1]

VON

TOMOWO ONO.

(Biologisches Institut der Kaiserlichen Tôhoku Universität, Sendai.)

Über die Liliaceen gibt es eine beträchtliche Anzahl embryologischer Arbeiten. Die Embryosackbildung dieser Familie zeigt starke Variationen, denn *Normal-*, *Codiaeum-*, *Scilla-*, und *Lilium*-Typus sind bei dieser Familie nebeneinander gefunden worden. Dagegen sind betreffs der Endospermbildung bis vor kurzem nur nukleäre Typen entdeckt worden (SCHÜRHOFF, 1924). Durch neuere Arbeiten von SEELJEB (1924), ONO (1926, 1928[2]), SCHNARF (1928 a, b) und STENAR (1928 a, b) ist aber gezeigt worden, dass bei den *Melanthioideae,* *Asphodeloideae, Lilioideae, Asparagoideae* und *Aletroideae* der betreffenden Familie ein helobiales Endosperm vorhanden ist. Das Vorkommen des helobialen Endosperms, mit dem eine Zwischenform des zellulären und nukleären Endosperms bezeichnet wird, ist für die Phylogenie und das System der *Liliaceae* sehr bedeutungsvoll. Um das oben erwähnte Verhältnis zu klären, sind aber noch viele Untersuchungen notwendig. Zu diesem Zweck hat Autor im Laufe der letzten Jahre einige embryologische Beobachtungen an einer Anzahl von Arten der Liliaceen gemacht.

Die Materialien wurden hauptsächlich in BOUINS Flüssigkeit fixiert. Zur Färbung wurde HEIDENHAINS Eisen-Hämatoxylin od. Safranin-Lichtgrün benutzt.

Melanthioideae.

Im Jahre 1926 habe ich schon eine embryologische Arbeit über *Heloniopsis breviscapa*, die zu den *Melanthioideae* gehört, veröffentlicht.

[1] Nach ENGLER, 1888. [2] Vorläufige Mitteilung „ Endospermbildung von Liliaceen ".- Bot. Mag., TOKYO, 42:445-449.

Die bei dieser Unterfamilie ausgeführten embryologischen Untersuch-
ungen seien in unten stehender Tabelle zusammengestellt:

	Typus des Embryosacks[1]	Typus des Endosperms[2]
Tofieldia calyculata (SEELIEB, 1924)	N	(H)
Heloniopsis breviscapa (ONO, 1926)	N	(H)
Zygadenus elegans (STENAR, 1928 a)	N	—
Veratrum album (STENAR, 1928 a)	N	(H)
Gloriosa-Arten (AFZELIUS, 1918)	N	—
Tricyrtis hirta (IKEDA, 1902)	N	(N)
Colchicum autumnale (WINAWER, 1919)	N	(N)

Die Embryosackbildung aller dieser Arten geht normal vor sich,
während der Endospermbildungsmodus zum Teil nukleär und zum
Teil helobial ist.   In vorliegender Mitteilung möchte ich einige kürzlich
festgestellte oder nachgeprüfte embryologische Resultate bei den
japanischen Arten von *Tofieldia, Narthecium, Methanarthecium, Vera-
trum* und *Colchicum,* hinzufügen.

Tofieldia japonica, MIQ.

SEELIEB (1924) hat bei *Tofieldia calyculata* festgestellt, dass die
Embryosackbildung normal und die Endospermbildung helobial ist.
Bei *T. japonica* verläuft die Embryosackbildung auch normal.   Nach
der Reduktionsteilung werden aus der Embryosackmutterzelle vier in
einer Linie liegende Megasporen.   Die innerste entwickelt sich zum
8-kernigen normalen Embryosack.   Der Zentralkern des Embryosacks
liegt stets in der Nähe der Antipoden.   Dies ist eine normale Er-
scheinung bei den Helobiaetypus-Endosperm zeigenden Pflanzen.   Fig.
1 stellt einen Embryosack dar, bei dem die Befruchtung vollendet ist.
Der primäre Endospermkern teilt sich an derselben Stelle, und der
Embryosack wird in zwei ungleich grosse Kammern, die obere, grosse,
mikropylare und die untere, kleine, chalazale Kammer, geteilt (Fig.
2, 3).   Die weitere Entwicklung der beiden Kammern zeigen Figg.
4–6.   Fig. 4 stellt ein etwas entwickeltes Endosperm dar, das durch
eine dünne Membran in zwei Kammern geteilt ist.   Bei *Heloniopsis
breviscapa* (ONO, 1926), einer nahe verwandten Art, ist diese Membran

---

[1] N bedeutet Normaltypus.   [2] (H) bedeutet helobiales, (N) nukleäres Endosperm.

nicht zu sehen; beide Kammern sind nur durch eine Hautschicht voneinander geschieden. In beiden Kammern erfolgen dann freie
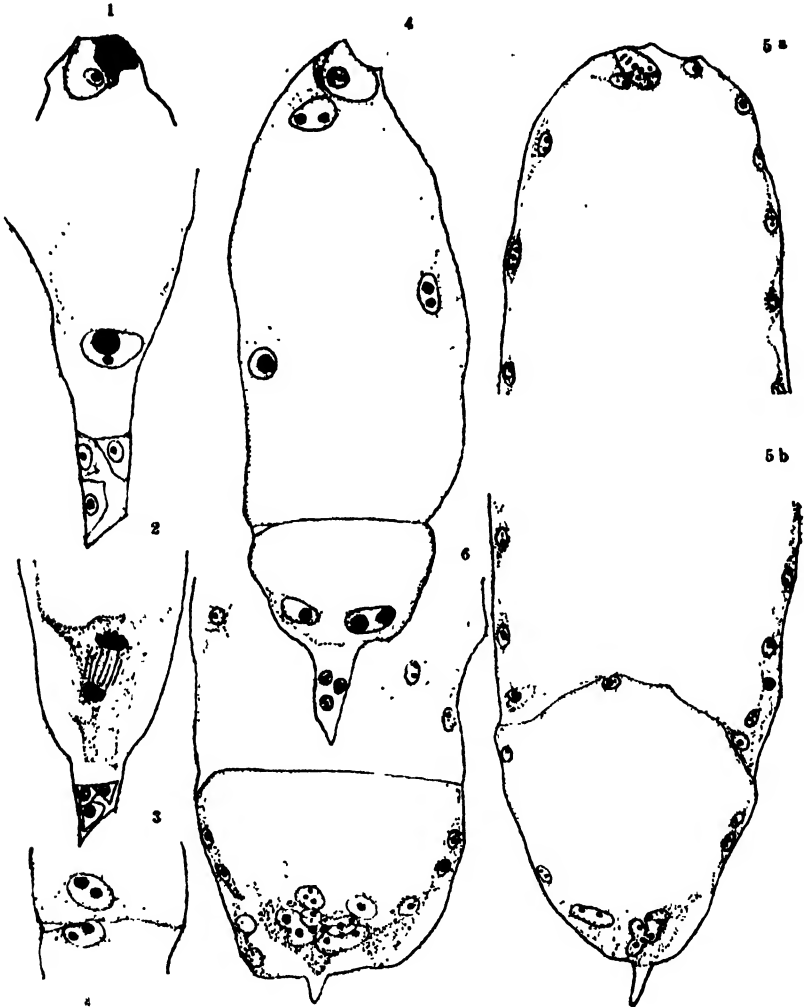
Tofieldia japonica. Fig. 1-6. Endospermbildung. Fig. 1-4 (×600). Fig. 5-6 (×300).

Kernteilungen (Fig. 5 a, b). Das Plasma in der oberen Kammer ist zuerst dünn und gleichmässig verteilt, während das der unteren Kam-

mer öfters im Basalteil viel mehr zusammengehäuft und mit einigen hypertrophierten Kernen ausgestattet ist. Solche Plasmaansammlung ist besonders klar bei Fig. 6 zu beobachten. Vielzellbildung kommt dann im Plasmawandbelag beider Kammern vor, und die Zellen vermehren sich zentripetal immer mehr. Im fast reifen Stadium findet sich sogar homogenes Endospermgewebe, und eine Begrenzung beider Kammern ist nicht zu sehen.

Narthecium asiaticum, MAXIM.

Die Embryosackmutterzelle von *N. asiaticum* ist oben durch eine Zellschicht von der Nuzellusepidermis getrennt (Fig. 7). Nach der Reduktionsteilung treten die vier normalen Megasporen auf, und der achtkernige Embryosack entwickelt sich aus der chalazalen Zelle (Fig. 8–14). Die Verschmelzung der zwei Polkerne findet im unteren Teil des Embryosacks statt (Fig. 15), ganz wie bei anderen Helobiaetypus-Endosperm zeigenden Arten. Fig. 16 stellt einen fertigen Embryosack dar. Die Eizelle im Eiapparat des befruchtungsreifen Sacks zeigt nichts Besonderes. Die Synergiden erscheinen dichter und absorbieren den Farbstoff intensiver. Am oberen Ende der Synergiden befindet sich je eine Vakuole. Der grosse Zentralkern des Embryosacks liegt in der Nähe der Antipoden. Die 3 Antipoden sind einkernig und liegen übereinander im taschenförmig auslaufenden Ende des Embryosacks. Der Befruchtungsvorgang wurde nicht beobachtet. Die erste Teilung des primären Endospermkerns findet im unteren Teil des Embryosacks statt, dann wird der Embryosack in zwei ungleich grosse Kammern geteilt, eine mikropylare, grosse und eine chalazale, kleine (Fig. 17, 18). Die beiden Kammern sind durch eine Hautschicht von Plasma getrennt, die aber keine Zellulosemembran darstellt. Im Plasmawandbelag der oberen Kammer erfolgen dann rasch sich wiederholende freie Kernteilungen. Zuletzt wird von der oberen Kammer ein Hauptendospermgewebe gebildet. In der unteren Kammer kommen auch mehrmalige Teilungen vor. Fig. 23 zeigt ein weiter entwickeltes Stadium. Im fast reifen Endosperm ist die Basalkammer völlig mit dichter Plasmamasse erfüllt, und diese wird zunächst durch einige grosse Zellen quer geteilt. In jeder sieht man einzelne Kerne, welche durch Farbstoffe stark gefärbt werden. Fig. 24 a und 24 b zeigen zwei aufeinanderfolgende Schnittserien der fast reifen Basalkammer,

wo im Zentrum der oberen Zellen viele gedrängte Kerne zu sehen
sind.
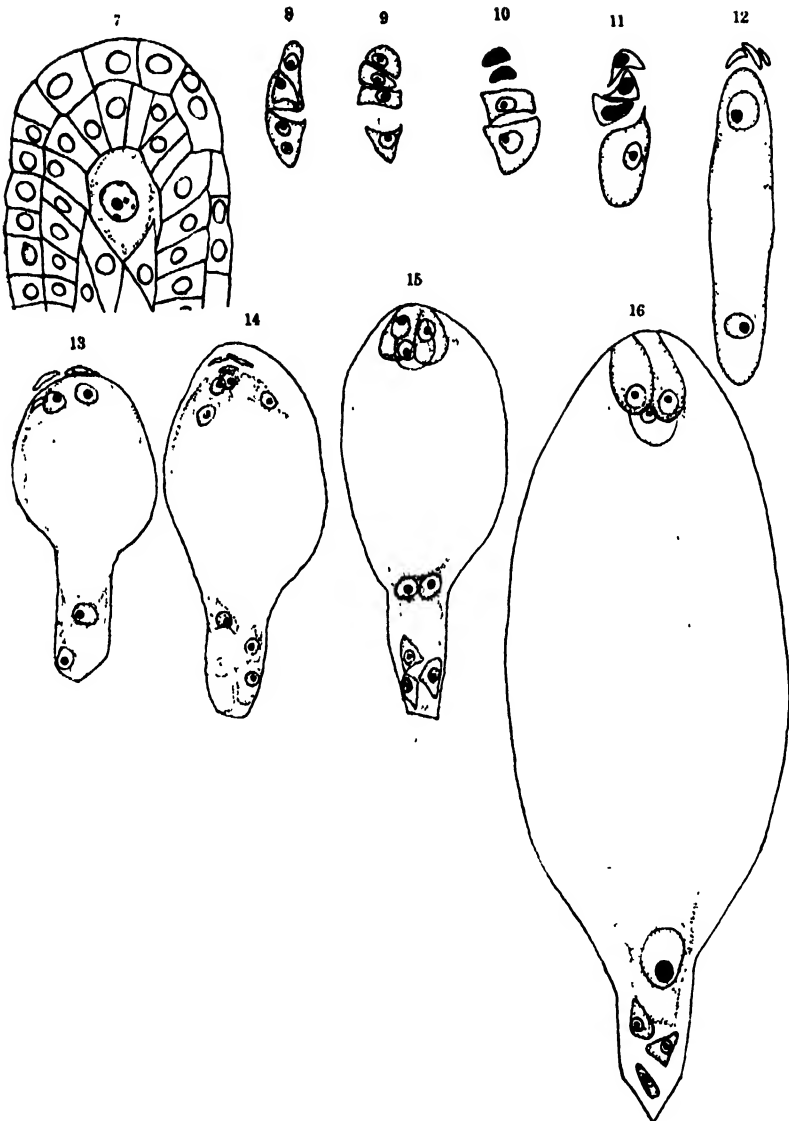
Narthecium asiaticum. Fig. 7-16. Embryosackbildung (× 600).
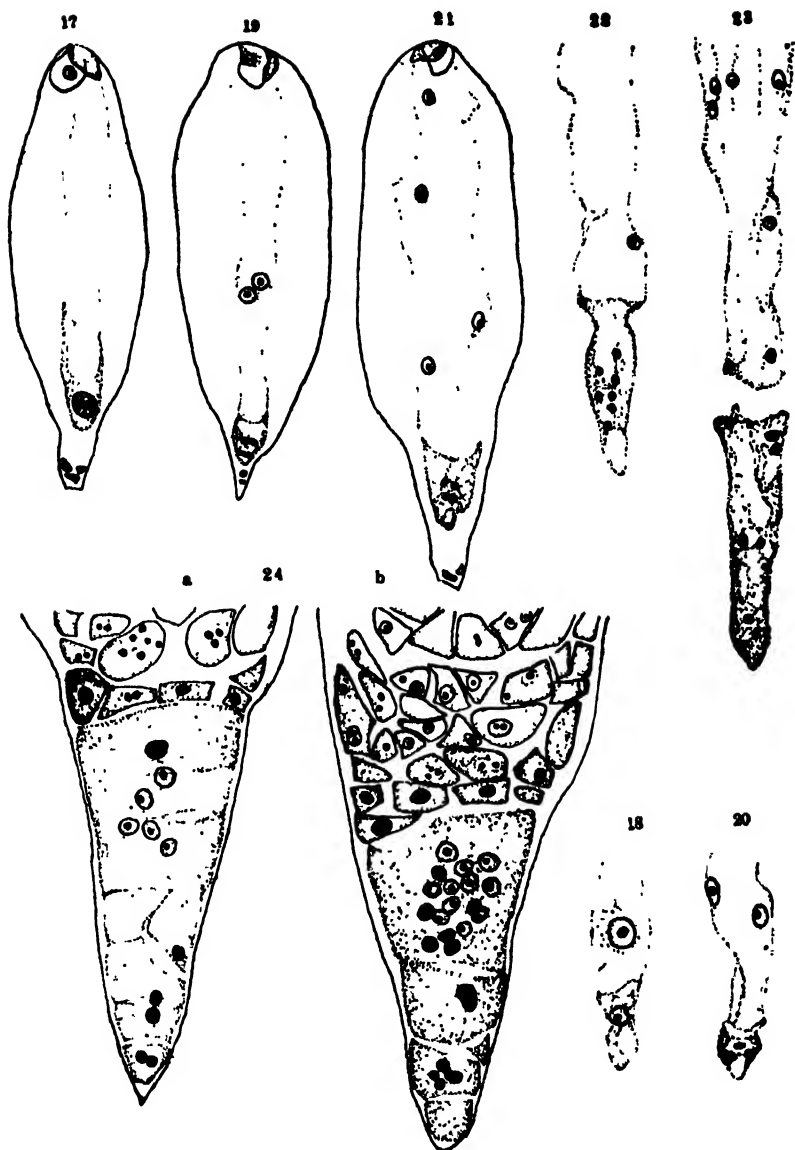
Narthecium asiaticum. Fig. 17–24. Endospermbildung (×180).
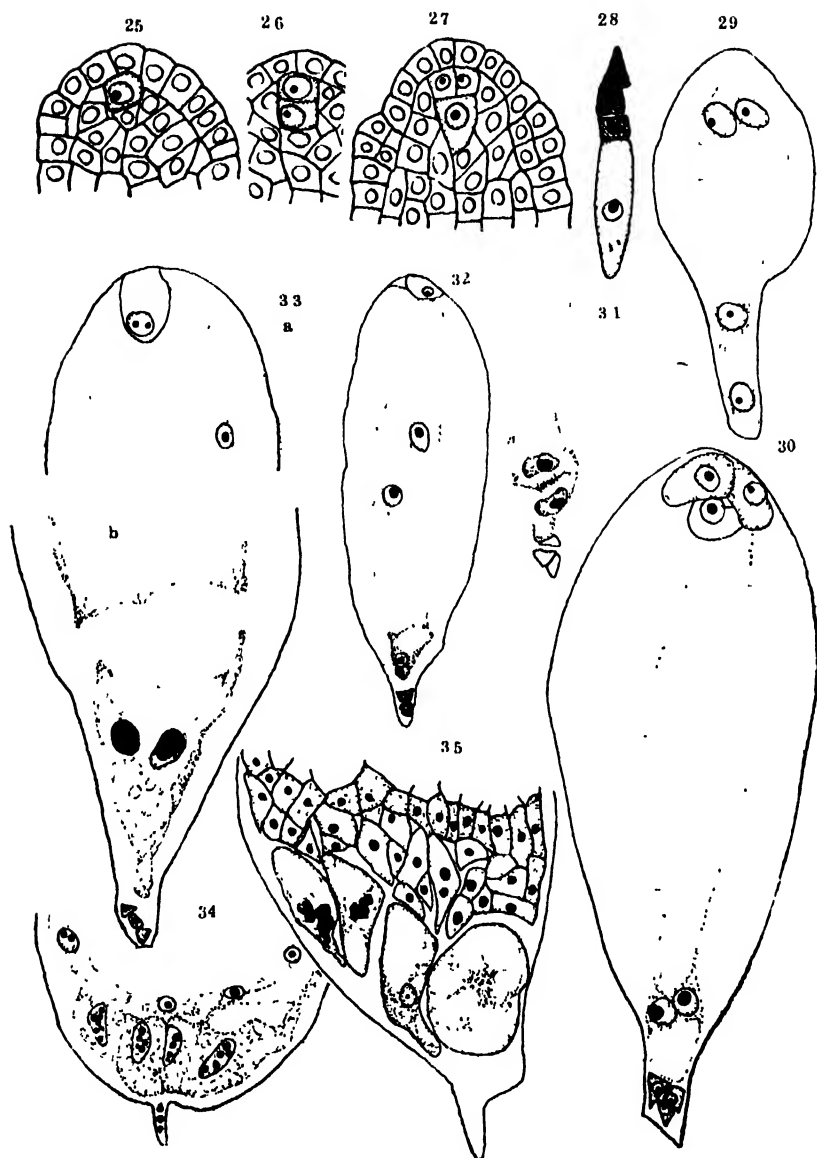
Metanarthecium luteo-virde. Fig. 25-30. Embryosackbildung (×600). Fig. 31-35. Endospermbildung. Fig. 31, 33-35 (×180), Fig. 32 (×300).

Metanarthecium luteo-virde, MAXIM.

Die Archesporzelle von *M. luteo-virde* tritt als verhältnismässig grosse Subepidermiszelle am Scheitel des Nuzellus auf. Nach der Teilung der Zelle wird die obere zur Deckzelle und die untere zur Embryosackmutterzelle (Fig. 25-27). Die Entwicklung des Embryosacks verläuft ganz normal, was schon bei anderen Melanthioideen nachgewiesen wurde (Fig. 28-30). Die Polkerne vereinigen sich verhältnismässig früh zu einem grossen Zentralkern, der in den unteren Teil des Embryosacks zu liegen kommt. Leider habe ich die Befruchtungserscheinung nicht gesehen. Die erste Teilungsspindel des primären Endospermkerns liegt parallel den Achsen des Embryosacks in seinem unteren Teil. Zwei ungleich grosse Kammern werden dann gebildet (Fig. 31). Zwischen den Kammern zeigt sich keine echte Zellwand (Fig. 32). Fig. 33 zeigt ein weiter entwickeltes Stadium des Endosperms; die obere Kammer ist gewöhnlich nukleär, während die chalazale eine zweikernige, dichte Plasmammasse darstellt. Der Kern der unteren Kammer teilt sich mehrmals. Wenn der Embryo eine vielzellige Kugel geworden ist, wird die untere Kammer wie in Fig. 34; in dichter Plasmamasse ordnen sich einige spindelförmige Kerne in der Richtung der Längsachse des Embryosacks, und in der oberen Kammer tritt Vielzellbildung ein, während die Zellen der unteren Kammer immer mehr vakuolisieren und die Kerne auch Anzeichen von Degeneration zeigen (Fig. 35).

Veratrum Maackii, REGEL. und *V. album*, L. var. *lobelianum*, BAK.

Von den *Veratrum*-Arten habe ich *V. Maackii* und *V. album* var. *lobelianum* untersucht. Die Embryosackbildung bei *V. Maackii* geht normal vor sich, wie die bei *V. album*, die STENAR (1928 a) geschildert hat. Die embryologischen Einzelheiten dieser Pflanze zeigen Figg. 36–43. Der Embryosack bei *Veratrum* ist sehr viel grösser als der bei den oben erwähnten Melanthioideen (Fig. 41). Der Eiapparat zeigt gewöhnliche Gestalt. Die Antipoden sind plasmareich und häufig 2- oder 4-kernig (Fig. 42). Das Vorkommen mehrkerniger Antipoden bei *Veratrum* und bei anderen Liliaceen (z. B. *Gloriosa*, *Heloniopsis*, und *Zygadenus*) ist schon seit langem bekannt. Der Endosperment-

wicklungsmodus ist helobial; der Embryosack ist in eine grosse mikropylare und eine kleine antipodale Kammer geteilt. Zwischen den beiden Kammern tritt keine feste Zellwand auf (Fig. 45). In dem Plasmawandbelag der beiden Kammern gehen dann freie Kernteilungen vor sich (Fig. 43). Die Zellbildung tritt zuerst in der oberen Kammer ein und später auch in der unteren.

Bei *V. album* var. *lobelianum* habe ich die jüngsten Stadien der Embryosackbildung nicht beobachtet, aber es dürfte kaum zu bezweifeln sein, dass der Embryosackbildungsmodus normal ist, wie bei *V. Maackii* und *V. album*. Fig. 44 zeigt einen reifen Embryosack. Die Antipoden sind zuerst einkernig und werden später zweikernig. Doppelte Befruchtung kommt bei dieser Pflanze vor (Fig. 45). STENAR
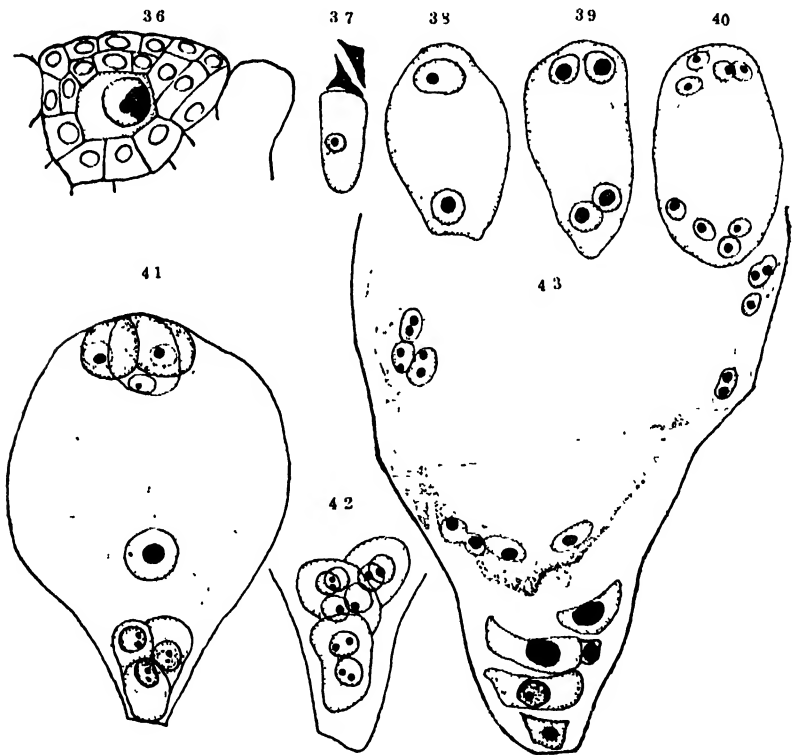
Veratrum Maackii. Fig. 36–41. Embryosackbildung (×450). Fig. 42. 2- od. 4-kernige Antipoden (×450). Fig. 43. Junges helobiales Endosperm (×300).

(1928) hat seinerzeit bei *V. album* das jüngste Endospermstadium
nicht beobachtet. Die vorliegenden Figuren 46 und 47 bei *V. album*
var. *lobelianum* bieten einen Ersatz dafür. Die erste Teilung des
primären Endospermkerns erfolgt parallel zur Embryosackachse, zwi-
schen den zwei gegliederten Kammern entsteht nie eine feste Zell-
membran. Die zweiten Teilungsspindeln treten sogar senkrecht zuein-
ander auf (Fig. 47). Fig. 48 zeigt einen weiter entwickelten Embryo-
sack, dessen Eizelle zweizellig ist, und in dessen oberer wie unterer
Kammer sich etwa 6 freie Kerne befinden. Das Plasma der basalen
Kammer ist viel plasmareicher als das der oberen, und im Zentrum
der Kammer befindet sich eine Vakuole. Die weitere Entwicklung
erfolgt fast wie bei *V. album* von STENAR (1928 a).
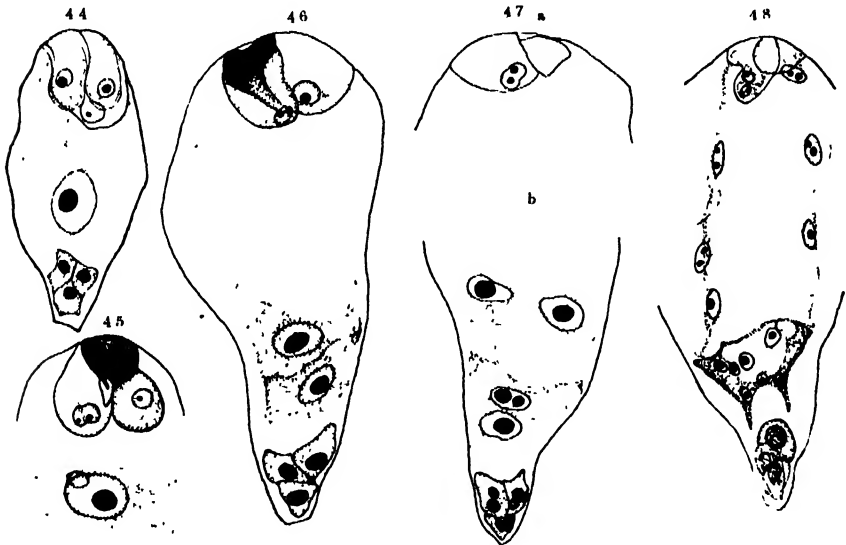
Veratrum album var. *lobelianum*. Fig. 44. Fertiger Embryosack (×225).
Fig. 45. Doppelbefruchtung (×225). Fig. 45-48. Endospermbildung (×225).

Tricyrtis hirta, HOOK. und *T. latifolia*, MAXIM.

Die Entwicklungsgeschichte bei *T. hirta* wurde schon von IKEDA
(1902) untersucht. Die Ergebnisse dieses Autors stimmen mit denen
meiner eigenen Untersuchungen über *T. hirta* und *T. latifolia* überein.
Von den vier Makrosporen entwickelt sich die unterste zum acht-

kernigen normalen Embryosack. Beim früheren Stadium der Endo-
spermbildung findet man den ganzen Embryosack voll von Plasma
und mit vielen freien Kernen. Die Zellteilung tritt da erst später
ein, was also sehr ähnlich wie bei *Elanthis* von DAHLGREN (1924) ist.

Colchicum autumnale, L.

HEIMANN-WINAWER (1919[1]) hat diese Pflanze schon embryologisch
untersucht und mitgeteilt, dass der achtkernige Embryosack normal
gebildet wird und die Endospermbildung nukleär ist. Ich habe die
Endospermbildung dieser Pflanze nochmal untersucht. Das Material
stammt aus dem Botanischen Garten zu TOKYO. Fixierung geschah
im Frühling. Ein schönes, nukleäres Endosperm zeigte sich; im
unteren Teil des Embryosacks wurde nie eine Basalkammer beobachtet.

Aletroideae.

Aletris foliata, FRANCH.

In der Literatur finden sich keine Angaben über die Embryologie
bei *Aletroideae*. Als Vertreter dieser Unterfamilie habe ich *Aletris
foliata* untersucht. Der Fruchtknoten dieser Pflanze ist dreifächerig,
und oberhalb des Fruchtknotens finden sich viele Drüsenzellen (Fig.
57). Die Samenanlage ist crassinuzellat; oberhalb der grossen Em-
bryosackmutterzelle findet sich eine einzige Zellschicht von Deckzellen
(Fig. 49). Der Embryosack wird nach dem Normaltypus gebildet,
d. h. die basale Tetradenzelle verdrängt die oberen und wird zum
achtkernigen Embryosack (Fig. 50–53). Fig. 54 zeigt einen fertigen
Embryosack. Die Vereinigung der Polkerne findet im unteren Teil
des Embryosacks statt, und daher liegt der Zentralkern des Embryo-
sacks stets an derselben Stelle. Einmal habe ich einen wurmförmigen
Spermakern neben dem Zentralkern gesehen. Der Endospermbildungs-
modus ist helobial. Die erste Teilungsspindel des primären Endo-
spermkerns kommt im unteren Teil des Embryosacks vor. Um die
Spindel findet sich reichlich Plasma, im übrigen aber ist der Embryo-
sack ziemlich plasmaarm. In der Anaphase der Kernteilung treten
Phragmoplasten auf, aber zwischen den dadurch geteilten zwei Zellen

---

[1] Zitiert nach SCHÜRHOFF (1926) und SCHNARF (1928).
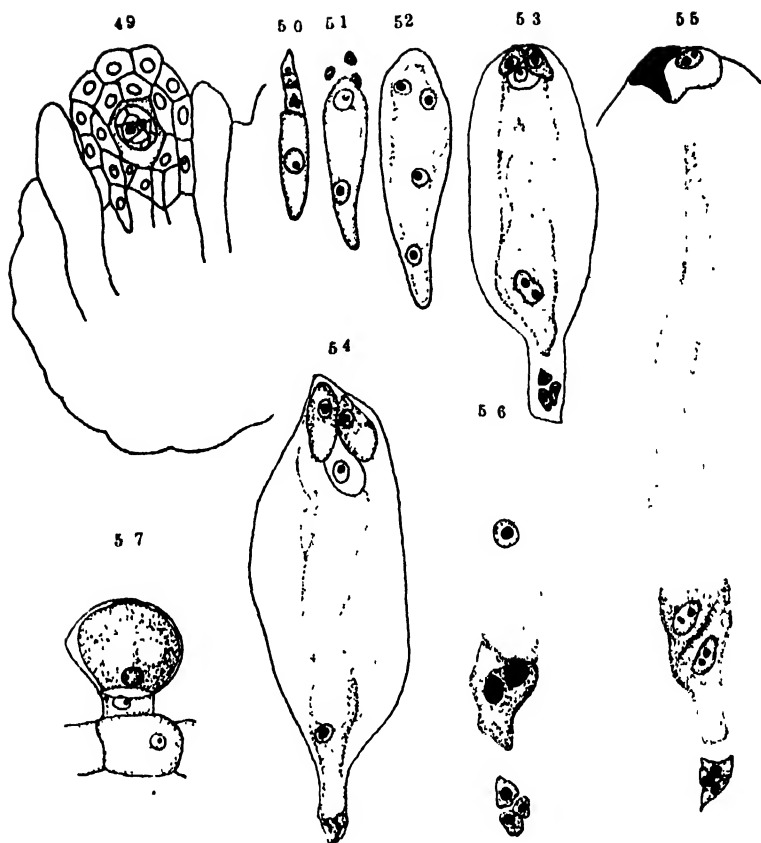
Aletris foliata. Fig. 49-54. Embryosackbildung (×300). Fig. 55-56. Endospermbildung (×300). Fig. 57. Eine Drüsenzelle (×600).

wird keine echte Zellulosemembran gebildet. Im Kern der oberen Zelle erfolgen wiederholt freie Kernteilungen im Plasmawandbelag. Die basale Zelle zeigt dann aber Anzeichen von Degeneration. Leider habe ich keine weitere Entwicklungsstadien erhalten.

Die oben geschilderten embryologischen Resultate bei einigen Pflanzen von *Melanthioideae* und *Aletroideae* seien unten als Tabelle zusammengestellt :

Melanthioideae	Typus des Embryosacks	Typus des Endosperms
Tofieldia japonica, Miq.	N	(H)
Narthecium asiaticum, Maxim.	N	(H)
Metanarthecium luteo-virde, Maxim.	N	(H)
Veratrum Maackii, Regel.	N	(H)
V. album, L. var. *lobelianum*, Bak.	—	(H)
Tricyrtis hirta, Hook.	N	(N)
T. latifolia, Maxim.	N	(N)
Colchicum autumnale, L.	-	(N)
Aletroideae		
Aletris foliata, Franch.	N	(H)

LITERATURVERZEICHNIS

Afzelius, K. 1918.   Zur Entwicklungsgeschichte der Gattung *Gloriosa*. Acta Horti Bergiani, Bd. 6, Nr. 3:3-12.

Engler, A. 1888.   Die natürlichen Pflanzenfamilien. Leipzig.

Ikeda, T. 1902.   Studies in the physiological functions of the antipodals and related phenomena of the fertilization in *Liliaceae*. I *Tricyrtis hirta*. Bull Coll. Agr., Univ. Tokyo, 5:41-71.

Ono, T. 1926.   Embryologische Studien an *Heloniopsis breviscapa*. Sci Rep., Tohoku Imp Univ., IV, 2.93-104

Ono, T. 1928.   Endospermbildung von Liliaceen (japanisch) Bot Mag Tokyo, 12 445-449.

Schnarf, K. 1928 a.   Über die Endospermentwicklung bei *Ornithogalum* Oestr Bot. Zeitschr., LXXVII:173-177.

Schnarf, K. 1928 b.   Über das Endospermhaustorium bei *Anthericum*. Oestr. Bot. Zeitschr., LXXVII:287-291.

Schnarf, K. 1928 c.   Embryologie der Angiospermen. Berlin.

Schürhoff, P. N. 1924.   Die Haploidgeneration der Blütenpflanzen. Bot. Jahrb. (Englfr), 59:198-293.

Schürhoff, P. N. 1926.   Die Zytologie der Blütenpflanzen. Stuttgart.

Seklieb, W. 1924   Beträge zur Entwicklungsgeschichte von *Tofieldia calyculata* (L.) Wahlenb. Bot. Not., 172-178.

Stenar, H. 1928 a.   Zur Embryologie der *Veratrum*- und *Anthericum*-Gruppen Bot. Not., 5-6:357-387.

Stenar, H. 1928 b.   Zur Embryologie der *Asphodeline*-Gruppe. Ein Beitrag zur systematischen Stellung der Gattung *Blubine* und *Paradisea*. Sv. Bot. Tidskr., 22:145-156.

Studies on the Hepaticae of Japan. II.

BY

YOSHIWO HORIKAWA.

(Biological Institute, Tôhoku Imperial University, Sendai.)
(With Plates XVI–XVIII & 15 Text-Figures.)

Genus : FIMBRIARIA NEES (1820)

Syn. *Asterella* PAL. BEAUV. (1810)

Hypenantron CORDA (1829)

Fimbriaria Yoshinagana HORIKAWA, sp. nov.
(Pl. XVI.)

Dioica, mediocris, valde flaccida, dilute viridis, lucida, margine posticeque, purpurea, tenuis. Frons ad 20 mm. longa et 4 mm. lata, dichotoma, plana margine ±undulata, apice emarginata ; alae tenerrimae. Costa fronde 6-plo angustior postice late convexa, alis ubique aequicrassis, margine unicellulas latis. Stratum anticum costae duplo humilius in medio, cavernis amplis ; fila libera nulla. Stomata parum convexa, poro 33 μ in diametro, 6 cellulis biseriatis formata. Cellulae epidermidis haud incrassatae, tenerae, trigonis parvis incrassatis. Squamae posticae remotae, parvae, purpureae, late lunatae, appendiculo longe ligulato apice acuto vel obtuso. Carpocephala parva, 3–4 mm. in diametro, disciformia, subhorizontalia, viridia, papulosa, lobis 2–4, obtusis ; perianthia oblongo-ovata, flava, subhorizontaliter patula, ad basin fere 7–8 fida ; capsula flavo brunnea. Pedunculus ad 10 mm. longus, validus, basi purpureus, superne stramineus, in sectione irregulariterque costatus, unisulcatus, basi et apice barbatus, paleis hyalinis sublinearibus. Sporae 78–92 μ in diametro, rufo-brunneae, reticulatim alatae, alis crenatis. Elateres 157–263 μ longi, 6.4–8.6 μ lati, flavo-brunnei, tenues et attenuati, spiris duplicatis filiformibus.

Fr. August.

Hab. On soil deposited in the cracks of sericite-schists in sunny places.

Loc.

Shikoku: Masunokawa-yama, Hongawa-mura, prov. Tosa (T. Yoshinaga, no. 2-type, Aug. 1928).

Distrib. This species endemic.

Dioicous. Xerophyte. Plants growing in extended pale green, shiny patches. Thallus 3-4 mm. broad and 12-20 mm. long, dichotomously branched, frequently overlapping, very tender, flat with somewhat undulate margins, the apex emarginate. Dorsal surface with somewhat distinct larger elongated areolae; epidermis composed of 5-7-angled cells with thin walls, mostly 30-34 μ wide (averaging 32 μ) and 46-63 μ long (averaging 54 μ), some cells containing oil-bodies. Rhizoids rather long, scanty, colourless, 15-21 μ in diameter, both smooth and tuberculate ones mingled. Midrib 6-times narrower than the thallus, convex below, usually the cells in the middle part with purple walls, rather suddenly passing into the 2-celled layered lamina, ending in a 1-celled margin. Antical layer low, about half the thickness of midrib, lacunae rather well developed, spaces not high without filaments. Pores simple, slightly elevated, mostly 33 μ in diameter, with 2 concentric rings, each ring being composed of 6 cells. Ventral scales distant, tender, in one row on each side of the midrib, purple, broadly lunate, appendage ligulate in outline, acute or obtuse at the apex. ♀- receptacle small, 3-4 mm. in diameter, disciform, green and papilose, composed of 2-4 (usually 4) horizontal, obtuse lobes; perianth arising from the ventral surface of the receptacle-lobes, deeply divided into 7-8 pointed and yellowish lobules, each enclosing a single capsule; capsule dehiscing at maturity longitudinally by irregularly brownish-yellow valves. Capsule-wall consisting a single layer of cells without annular thickenings. Peduncle of ♀ receptacle from the apex of a branch, 6-10 mm. long, valid with one ventral furrow, purple at lower part, surrounded by sublinear hairs on both lower and upper ends. Spores 78-92 μ in diameter, reddish-brown, regularly or somewhat irregularly reticulate, the lamellae low, the areolae 5-6-angled; margin paler in colour, 10-11 μ broad. Elaters 157-263 μ long and 6.4-8.6 μ wide, slender and bended, with 2 narrow brownish-yellow spires; rarely branched.

Genus: **CONOCEPHALUS** NECKER (1791)

Conocephalus NECKER, Element. Bot. III, p. 344 (1791); SCHIFFNER, in ENGLER u. PRANTL, Natürl. Pflanzenfam. Teil I, Abt 3, p 34 (1893); STEPHANI, Spec Hepat. Vol. I, p. 214 (1899).

Fegatella RADDI, Opusc. scientif. di Bologna II, p. 356 (1818); MÜLLER, in RABENHORST's Kryptogamen-Flora, Bd. 6, Abt. 1, p. 280 (1907)

Thallus dichotomously branched or alternately pinnate; areolae distinct, mostly hexagonal, stomata simple and elevated. Photosynthetic layers low, terminal cell of the chlorophyll-bearing filaments in these layers colourless, forming a beak. Peduncle of ♀ receptacle from the apex of a branch, long with a single rhizoid-furrow; ♀ receptacle obtusely conical or campanulate, almost entire, composed of 5–8 tubular involucres, each enclosing a single sporogonium. Perianth absent. Capsule with rather long pedicel, clavate-pyriform, dehiscing at maturity by throwing off an apical cap, the remainder splitting longitudinally. Spores large, papillose, multi-cellular, beginning to germinate while still within the capsule. Elaters 2–4-spiral, bluntly fusiform. ♂ receptacle disciform, sessile at apex of a branch, later becoming more elongated, surrounded by the dorsal layers of the thallus.

The following two species have been recognized in this genus.

Thallus very large, perennial and dark green; areolae large; no collar-shaped fold surrounding the base of the peduncle of ♀ receptale; ♂ receptacle disciform; gemmae absent ..*C. conicus*

Thallus smaller, annual and yellowish-green; areolae smaller; collar-shaped fold at the base of the peduncle of ♀ receptacle; ♂ receptacle more elongated; gemmae abundant in autumn....*C. supradecompositus.*

Conocephalus conicus (L.) NECKER
(Text-Fig. 1)

Marchantia conica LINNÉ, Spec. plant. II, p 1604 (1763).

Conocephalus conicus NECKER, Elem. Bot. III, p. 344 (1791); MIYAKE, in MATSUMURA et MIYOSHI, Cryptog. Jap. Vol. I, No. 4, Pl. XX (1899).

Conocephalus vulgaris BISCHOFF, Nova Acta Acad. Caes. Leop. Carol XVII, 2.

Conocephalus nemorosus HÜBENER, Hepaticol Germ., p 9 (1834).

Fegatella officinalis RADDI, Opusc. scientif. di Bologna II, p. 356 (1818)

Fegatella conica CORDA, Opiz, Beitr. 1, p. 649 (1829); TSUGE, Hepat. Jap. (MSS.) p. 30, Pl. XV (1887).

Hepatica conica LINDBERG, Hepat. utveckl. p. 5 (1877).

Dioicous. Mesophyte or hygrophyte. Growing in extended dark green, shiny layers. Thallus 0.8–2 cm. broad and 11–23 cm. long, dichotomously branched, firm and leathery, sometimes more or less pigmented with purple on lower surface, flat with somewhat undulate margins, the apex emarginate. Dorsal surface with distinct large areolae forming a regular network; epidermis composed of 5–7-angled cells with distinct trigones, mostly 30–51 μ wide (averaging about 38 μ) and 61–110 μ long (averaging about 87 μ). Pores (with their surrounding cells) simple, elevated, visible to the naked eye, mostly 184–302 μ wide and 302–368 μ long, with 5–6 concentric rings, each ring usually being composed of 6–7 cells. Rhizoids numerous, colourless and smooth. Air-chambers low, their boundaries distinct, occupying about 1/5 of thickness in the middle and almost all of it in the marginal portion, photosynthetic filament 2–5 celled, beak-cell narrower toward the apex. Midrib more or less prominent below; compact ventral tissue mostly 21–24 cells thick in the middle, oil-cells and mucilage-cells are well developed, some cells with distinct streak-shaped pits; somewhat suddenly passing into larger cells of the lamina, ending in a 1-celled margin. Ventral scales rather distant, tender, in one row on each side of the midrib, with a violet, reniform or orbicular appendage. ♀ receptacle obtusely conical, almost entire, brownish-yellow, composed of 5–8 tubular involucres, each enclosing a single sporogonium. Peduncle of ♀ receptacle from the apex of a branch, with a single rhizoid-furrow, 46–87 mm. long, hyaline except for the purple base. Capsule clavate-pyriform, apex rounded, dehiscing at maturity by throwing off an apical cap, the remainder splitting longitudinally by 8 reflexed valves. Capsule-wall of a single layer of cells except at the apex, with yellowish annular thickenings. Spores 92–132 μ in diameter (averaging 116 μ), brownish yellow, papillose. Elaters short and very variable in breadth, usually 10.5–25.5 μ broad and 144–328 μ long with 3–5 brownish-yellow spires. ♂ receptacle at first green, later purple, sessile and disciform, its longitudinal diameter parallel to the thallus-axis, surrounded by the dorsal layers of thallus. Gemmae absent.

Fr. April. Sexual organs ripen in May.

Hab. On moist rocks and soil in shady places.
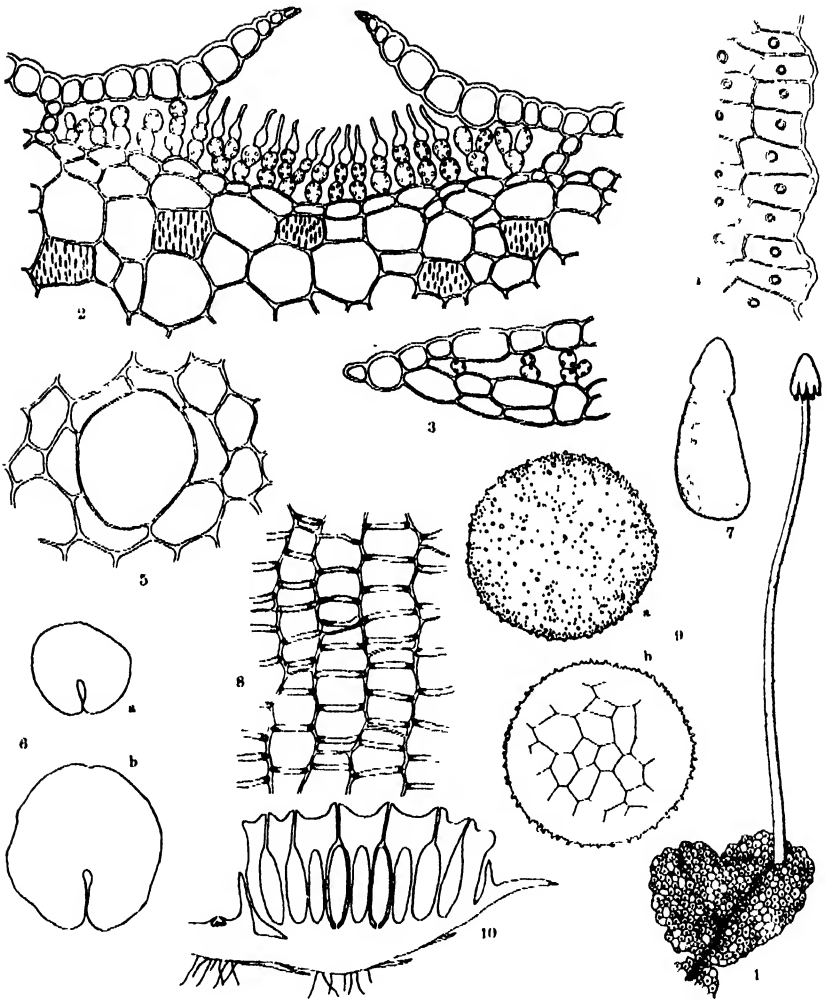
Text-Fig. 1. *Conocephalus conicus* (L.) NECKER

1. Part of thallus with mature sporophyte in natural size. 2. Cross section of part of thallus including a pore, ×144. 3. Marginal portion of thallus. in cross-section, ×144. 4. Marginal part of thallus, upper view, ×8. 5. Mucilage cell, in cross-section, ×233, 6. Cross-section of ♀ peduncle, ×12, a. near apex, b. near base. 7. Sporogonium, ×8. 8. Inner cell-wall of capsule, ×233 9. Spore, ×233, a. surface view, b. optical section. 10 ♂ receptacle, in longitudinal section, ×8.

Loc.

Hokkaido : Ônuma, prov. Oshima (Y. HORIKAWA, no. 1343, Nov. 1928).

Honshiu : Asamushi, prov. Mutsu (Y. HORIKAWA, no. 770, July 1927) ; Mt. Hakkôda (900 m., Shukayu), prov. Mutsu (Y. HORIKAWA, no. 1222, July 1928) ; Nohezi, prov. Mutsu (Y. HORIKAWA, no. 757, July 1927) ; Tsuta, prov. Rikuchû (Y. HORIKAWA, no. 1275, Sept. 1928) ; Hiraizumi, prov. Rikuchû (Y. HORIKAWA, no. 1184, May 1928) ; Sendai, prov. Rikuzen (Y. HORIKAWA, no. 260, March 1927) ; Deyu, Kitakanbara-gôri, prov. Echigo (M. SHIMAKURA, July 1927) ; Yûkyû, the hill, Koshi-gôri, prov. Echigo (M. SHIMAKURA, Sept. 1927) ; Mt. Kiyozumi, prov. Awa (T. MAKINO, Apr. 1896, in Herb. 2nd Higher School) ; Hakone, prov. Sagami (S. KOMATSU, Aug. 1906, in Herb. 2nd Higher School) ; Fukuosan, Mie-gôri, prov. Ise (Y. MURATA, in Herb. Imp. Univ. Tokyo, no. 6, Nov. 1911) ; Takahashi-machi, prov. Bitchu (M. INUMARU, no. 2, Nov. 1928) ; Tamakawa-mura, Kawakami-gôri, prov. Bitchu (M. INUMARU, no. 6, Dec. 1928) ; Hiroshima, prov. Aki (Y. HORIKAWA, no. 144, Feb. 1924) ; Insl. Miyajima, prov. Aki (Y. HORIKAWA, no. 645, Apr. 1927) ; Iwakuni, prov. Suou (Y. HORIKAWA, no. 286, March 1927).

Shikoku : Sakawa-machi, prov. Tosa (T. YOSHINAGA, no. 1245, July 1928).

Kiushiu : Aida, prov. Higo (K. MAYEBARA, no. 1, Feb. 1927) ; Aoshimamura, prov. Hiuga (Y. HORIKAWA, no. 358, Apr. 1927) ; Mt. Aoi-dake, prov. Hiuga (Y. HORIKAWA, no. 431, Apr. 1927) ; Mt. Kirishima, prov. Osumi (Y. HORIKAWA, no. 487, Apr. 1927) ; Kagoshima, prov. Satsuma (Y. HORIKAWA, no. 537 Apr. 1927).

Distrib. Europe, Siberia, Caucasus, Himalaya, China, Alaska, North America, and Azores, Madeira, Canary Islands.

Conocephalus supradecompositus (LINDB.) STEPHANI
(Text-Figs. 2–3)

Sandea supradecomposita LINDBERG, Acta Soc. F. Fl. Fenn., II, no. 5.

Conocephalus supradecompositus (LINDB.) STEPHANI, Bull. Herb. Boiss. Vol. V, p. 83 (1897); OKAMURA, in MAKINO's Nipp. Shokub. Dzukwan, p. 1254 (1925).

Fegatella sp. TSUGE, Hepat. Jap. (MSS.) p. 33 (1887).

Dioicous. Mesophyte. Growing in densely caespitose, yellowish

green patches. Thallus not shiny, 2-3 mm. broad and 25-30 mm. long, repeatedly dichotomous, sometimes alternately pinnate, somewhat firm, flat with dentate-lobed margin. Dorsal surface with indistinct small areolae forming a regular network; epidermis composed of 5–7-angled thin-walled cells, mostly 51–55 μ wide (averaging about 53 μ) and 74–104 μ long (averaging about 97 μ). Pores (with their surrounding cells) simple, elevated, visible to the naked eye, mostly 79-158 μ long and 66-118 μ broad, with 6-7 concentric rings of cells, the innermost ring composed of 6-7 cells. Rhizoids numerous, colourless and smooth. Air-chambers low, their boundaries distinct, usually occupying about

Text-Fig. 2. Growing-habit of *Conocephalus supradecompositus* (LINDB.) STEPHANI, bearing numerous mature ♂ receptacles in natural size.

1/6 of thickness in the middle and almost all of it in the marginal portion, photosynthetic filament 1-3 celled, beak-cell being broader. Midrib prominent below; compact ventral tissue mostly 13-16 cells thick in the median portion, mucilage-cells are well developed, some cells with distinct streak-shaped pits; somewhat suddenly passing into larger cells of the lamina, ending in 1- or 2-celled margin. Ventral scales rather distant, tender, in one row on each side of the midrib,

with a violet, orbicular appendage. Capsule clavate-pyriform, apex
pointed, dehiscing at maturity by throwing off an apical cap, the
remainder splitting longitudinally by irregularly 8 reflexed valves.
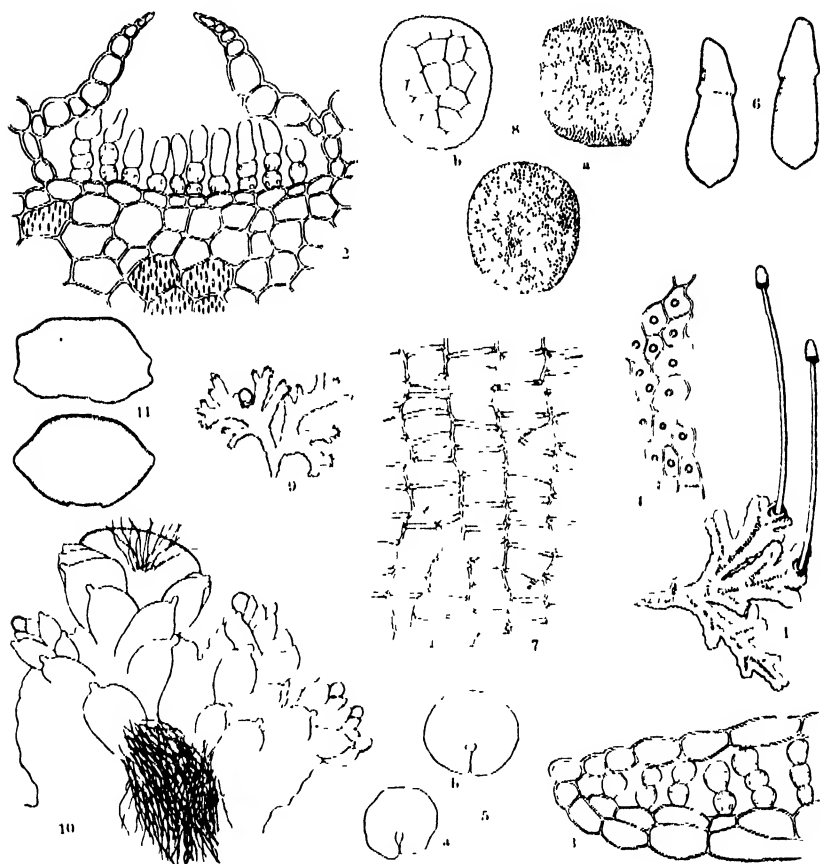
Text-Fig. 3. *Conocephalus supradecompositus* (LINDB.) STEPHANI

1. Habit of plant with mature sporophytes in natural size 2. Cross-section
of part of thallus including a pore, ×141 3. Marginal portion of thallus, in
cross-section, ×141 4. Marginal part of thallus, surface view, ×8. 5. Cross-
section of ♀ peduncle ×12, a. near apex, b. near base 6. Sporogonia, ×8 7.
Inner cell-wall of capsule, ×233. 8 Spores, ×233, a. surface view, b. optical
section. 9. Habit of plant with a young sporophyte and many gemmae-branches
in natural size. 10. A part of ditto, ventral view, ×12. 11. Gemmae, ×12.

Capsule-wall of a single layer of cells except at the apex, with brownish-yellow annular thickenings. ♀ receptacle campanulate, almost entire, brownish, composed of 1-8 tubular involucres, each enclosing a single sporogonium. Peduncle of ♀ receptacle from the apex of a branch, with a single rhizoid-furrow, 25-42 mm. long, hyaline but purple below. The base surrounded by collar-shaped elevated fold of the thallus. Spores 66-82.5 μ (averaging 72 μ) in diameter, yellowish-brown, minutely papillose. Elaters relatively short and variable in breadth, 8.5-21.3 μ broad, 52.5-289 μ long, with 2-5 yellowish spires. Gemmae are produced numerously in autumn at the top of short branches, lenticular, broader than long, dark purple.

Fr. April. Both ♀ and ♂ sexual organs are ripe in August.

Hab. On moist shady soil in gardens, banks and sides of ditches.

Loc.

Hondo: Asamushi, prov. Mutsu (Y. HORIKAWA, no. 771, July 1927); Nohezi, prov. Mutsu (Y. HORIKAWA, no. 711, July 1927); Sendai, prov. Rikuzen (Y. HORIKAWA, no. 261, March 1927 & no. 1161, May 1928); Isl. Katsura-shima, prov. Rikuzen (Y. HORIKAWA, no. 780, July 1927); Osawa-mura, prov. Rikuzen (M. SHIBATA, no. 1015, Oct. 1927); Nagaoka, prov. Echigo (M. SHIMAKURA, Sept. 1927); Tokyo (T. MAKINO, Sept. 1897, in Herb. 2nd Higher School); Mt. Kiyozumi, prov. Awa (S. KOMATSU, Aug. 1906, in Herb. 2nd Higher School); Hakone, prov. Sagami (A. YASUDA, Aug. 1905, in Herb. 2nd Higher School); Chikusa-mura, Mie-gôri, prov. Ise (Y. MURATA, in Herb. Imp. Univ. Tokyo, no. 5, Nov. 1911); Takahashi-machi, prov. Bitchu (M. INUMARU, no. 3, Nov. 1928); Hiroshima, prov. Aki (Y. HORIKAWA, no. 4, Sept. 1922).

Shikoku: Sakawa-machi, prov. Tosa (T. YOSHINAGA, no. 1247, July 1928); Kôchi, prov. Tosa (T. YOSHINAGA, no. 1246, July 1928).

Kiushiu: Kumamoto, prov. Higo (Y. HORIKAWA, no. 631, Apr. 1927); Aida, prov. Higo (K. MAYEBARA, no. 27, March 1927); Miyakonojô, prov. Hiuga (A. NOGUCHI, no. 1, Aug. 1927), Aoshima-mura, prov. Hiuga (Y. HORIKAWA, Apr. 1927); Kagoshima, prov. Satsuma (Y. HORIKAWA, no. 538, 573, Apr. 1927).

Distrib. China (prov. Shenshi).

Genus: **LUNULARIA** MICHELI (1729)

Lunularia cruciata (L.) DUMORTIER
(Text-Figs. 1-5)

Marchantia cruciata LINNÉ, Spec. plant II. p. 1604 (1763).

Lunularia vulgaris MICHELI, Nov. plant. gen., p. 4 (1729).

Lunularia cruciata (L.) DUM., SCHIFFNER, in ENGLER u. PRANTL, Natürl. Pflanzen-
fam., Teil I, Abt. 3, p. 35 (1893); STEPHANI, Spec. Hepat., Vol. I, p 143 (1899).

Dioicous. Mesophyte. In extended, dense, intense green and
shiny layers, becoming brownish-yellow with age. Thallus 0.4 -
0.8 cm. broad and 1.5-2 cm. long, obcordate and emarginate at the
apex, slightly concave, margin descending, sinuate. Dorsal epidermis
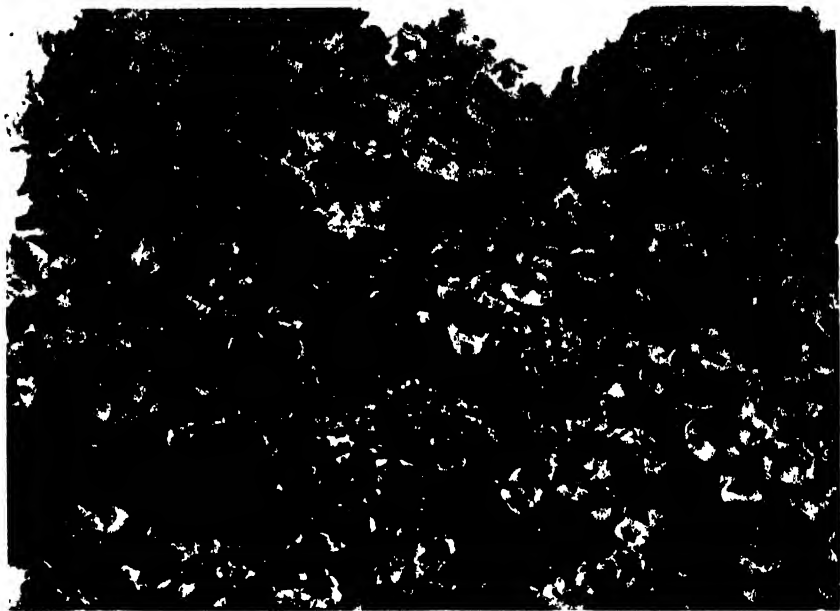
Text-Fig. 4. Growing-habit of *Lunularia cruciata* (L.) DUM., bearing numerous crescent-shaped cupules in which are produced gemmae in natural size.

composed of 5-7-angled cells with thickened or thin walls according
to the condition of habitat, mostly 17-23.4 μ wide (averaging about
21 μ) and 34-53 μ long (averaging about 42.5 μ). Pores (with their
surrounding cells) simple, highly elevated, mostly 78.8-98 μ wide and

105-131 μ long, with 4-5 concentric rings, each ring being usually composed of 6 hyaline cells. Rhizoids numerous and colourless, smooth or tuberculate. Air-chambers relatively well developed, their boundaries distinct, occupying about 1/5-1/4 of thickness in the middle and almost the whole in the marginal portion. Midrib more or less prominent below ; compact ventral tissue mostly 16-20 cells thick in the middle, oil-cells distinct and scattered, gradually passing into the lamina, ending in a 1-celled margin. Ventral surface green, scales in one row on
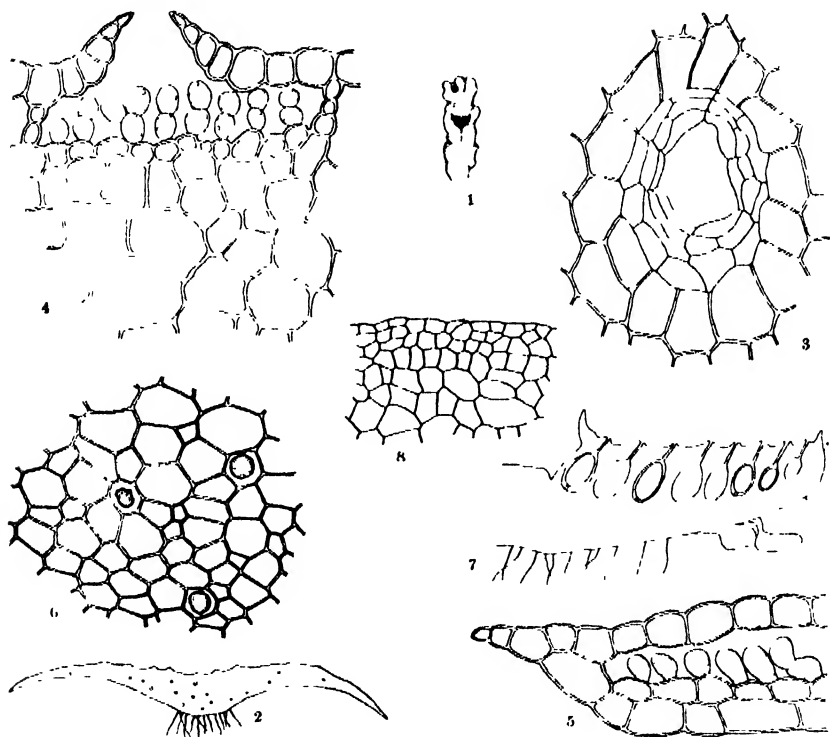
Text-Fig. 5. *Lunularia cruciata* (L.) Dumortier

1. A thallus with crescent-shaped cupule and ♂ receptacle, in natural size.
2. Cross-section of thallus, ×12. 3. A pore surface view, ×233. 4 Ditto, in
cross-section, ×233. 5. Cross-section of marginal portion of thallus, ×233. 6.
Cells from middle part of thallus including three oil-bodies, in cross-section,
×114. 7. Longitudinal-section of receptacle, ×16. 8. Marginal portion of cupule,
×114.

each side of the midrib, hyaline, very tender, broadly lunate with a rotundate appendage and several oil-cells. ⚤ receptacle sessile, disciform at the apex of a short branch, becoming apparently lateral, surrounded, except in front, by the elevated border of the thallus. Gemmae numerous in semi-lunular cupules having entire margin of a fold of the epidermis on the postical side, lenticular, vertically inserted, with a 1-celled hyaline pedicel and a closed sinus at each side.

Antheridia ripe in November-December.

Hab.   On moist soil in flower-pots and gardens.

Loc.

Hondo: Sendai, prov. Rikuzen (Y. HORIKAWA, no. 258, Feb. 1927); Koishikawa Botanical Garden, Tokyo (Y. HORIKAWA, Jan. 1928); Hiroshima, prov. Aki (Y. HORIKAWA, May 1923).

Distrib.   Europe, Persia, Africa, North America, South America, Australia and Azores, Madeira, Canary Islands.

Remarks.   This genus, being composed of a single species, can be easily recognized from the other closely related genera by the crescent-shaped cupules, which are almost always present. So far as the author has observed, in our country the plants are either ⚤ or sterile, and ♀ plants are yet undiscovered. The genus is new to Japan.

Genus: **CHOMIOCARPON** LINDBERG (1877)

Chomiocarpon quadratus (SCOP.) LINDBERG
(Text-Fig. 6)

Marchantia quadrata SCOPOLI, Fl. Carn., p. 120 (1760).

Marchantia commutata LINDENBERG, Syn. Hep., p. 101 (1829).

Preissia quadrata NEES, Eur. Leb. IV, p. 135 (1838).

Preissia commutata NEES. Eur. Leb. IV. p. 117 (1838); STEPHANI, Bull. Herb. Boiss. Vol. V, p. 82 (1897).

Chomiocarpon quadratus (SCOP.) LINDBERG, Hepat. utveckl. p. 6 (1877); SCHIFFNER, in ENGLER u. PRANTL, Natürl. Pflanzenfam. Teil I, Abt. 3, p. 36 (1893).

Dioicous or monoicous. Mesophyte. Growing in rather extended pale green patches. Thallus attaining to 25 mm. in length and about 8 mm. in breadth, ligulate, furcate, margin thin, crenulate and purple. Dorsal epidermis composed of 5–7-angled cells with thin walls, mostly 31–47 μ in diameter (averaging about 39 μ). Pores (with their surrounding cells) compound, small but distinct to the naked eye, usually

78-131 μ long and 64-96 μ wide, with 4-5 superimposed concentric rings of cells, each ring being usually composed of 4-6 cells. Rhizoids numerous, 15-32 μ wide, colourless, smooth, rarely mingled tuberculate ones. Air-chambers distinct, filled with chlorophyll-bearing filaments. Midrib prominent below; compact ventral tissue mostly 23-26 cells
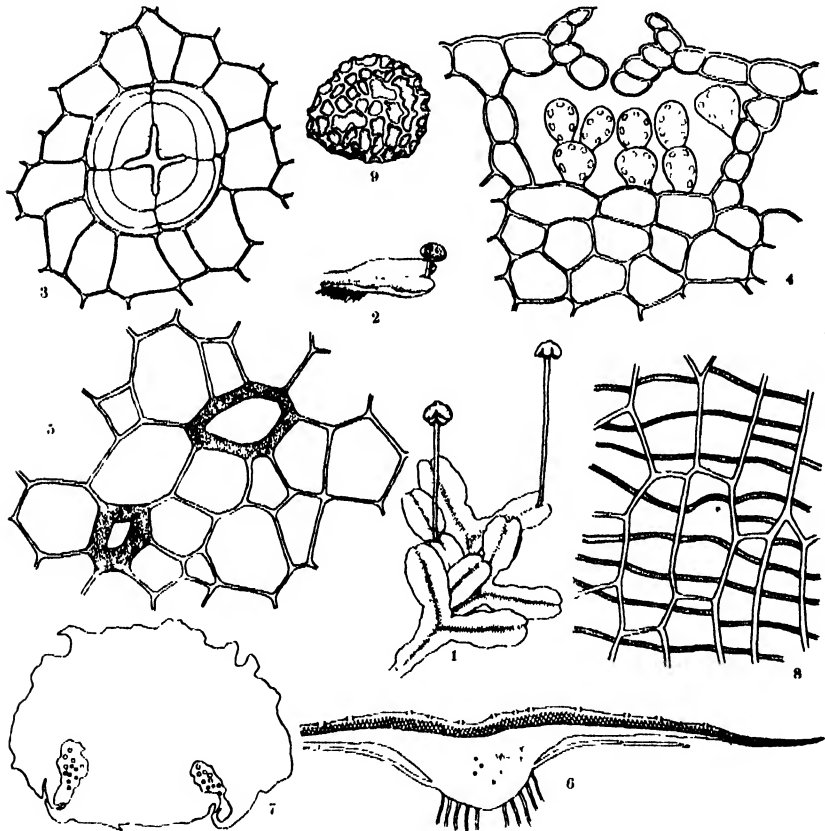
Text-Fig. 6. *Chomiocarpon quadratus* (Scop.) Lindberg

1. Habit of plant with mature sporophytes, in natural size. 2. A thallus with ♂ receptacle, in natural size. 3. A pore, in surface view, ×233. 4. Ditto, in cross-section, ×233. 5. Cells from the middle part of thallus including two sclerotic-fibres, in cross-section, ×460. 6. Cross-section of thallus, somewhat diagrammatized, ×16. 7. Peduncle of receptacle, in cross-section, ×33. 8. Inner-wall of capsule, ×233. 9. Spore, ×233.

thick in the median portion, the walls thin, some longitudinal brown
fibrous cells present, usually with 2 bundles of cells infected with
hyphae on each side of midrib, the cells here have purple walls, rather
suddenly passing into the lamina, ending in a 1-celled margin. Ventral
scales imbricate, in one row on each side of the midrib, pigmented
with purple, semi-lunar with a small lance-shaped appendage. ♀
receptacle hemispherical, green with short 4 cuneiform lobes, each
lobe with air-chambers and compound pores, one involucre under each
lobe, containing an inflated perianth with a single capsule. Capsule-
wall consisting of one layer of cells with numerous brown annular
thickenings. Capsule rather longly pedicellate, slightly exserted, sub-
globose, dehiscing at maturity by throwing off a cap, the remainder
splitting about to the middle by 6–7 irregular, revolute valves.
Peduncle of ♀ receptacle 20–25 mm. long with two rhizoid-furrows,
destitute of dorsal air-chambers. Spores brown, 45–60 μ (averaging
55 μ) in diameter, coarsely reticulate, rounded-tetrahedral, lamellae low,
forming numerous areolae, margin of spore appearing as if crenulate-
dentate. Elaters slender, 183–329 μ long, (abnormally 105 μ) and 8.5–
12.7 μ wide, with 2–3 reddish-brown loosely twisted spires. ♂ recep-
tacle 3 mm. wide, circular, upper surface green, scales rudimental or
indistinct on lower surface, rounded at the apex with thin wavy margin,
closely papillose, with compound pores and air-chambers. Cupules
absent.

Fr.   July-August.

Hab.   On moist soil and rocks in the subalpine districts.

Loc.

Honshiu :   Mt. Komagatake, prov. Ugo (I. KASHIMURA, no. 25,
July 1928) ; Mt. Iwate-san, prov. Rikuchû (S. HABARA, no. 1269, July
1928).

Distrib.   Europe, Spitzbergen, Siberia, Himalaya, Mexico, North
America, Alaska and Greenland.

Remarks.   The genus *Chomiocarpon* is monotypic. ♂ receptacle
of our Japanese specimens has a very short peduncle, compared with
the European ones.

Genus: **BLASIA** MICHELI (1729)

Blasia pusilla LINNÉ

(Text-Figs. 7–8, 10 & Tables 1 2)

Blasia pusilla LINNÉ. Spec plant. ed. I. p 1138 (1753); TSUGE, Hepat Jap (MSS.) p. 51 (1887), SCHIFFNER, in ENGLER u. PRANTL, Natürl Pflanzenfam Teil I, Abt 3. p. 57 (1893); STEPHANI, Spec Hepat Vol I, p 363 (1900).

Jungermannia Blasia HOOKER, Brit Jungerm Pl 83–84 (1816).

Dioicous. Mesophyte or hygrophyte. In yellowish green (frequently pigmented with purple) extended layers, sometimes forming rosettes. Thallus 3–5 mm. broad and 2 3 cm. long, repeatedly dichotomous, with several rotundate lobes, the margin ascending.   Midrib broad and nearly flat above and below, 4–10 cells thick in the median portion, usually with 4 longitudinal whitish bundles containing a deposit of $CaCO_3$, gradually passing into the 1-celled lobes, the marginal row of cells smaller, crenulate.   Lobes rather distant but distinctly succubous, alternate, more or less regularly arranged, semi-lunular in outline, $0.86–1.5 \times 1.7–2$ mm., the margin nearly entire. Cells of lobes 5–7-angled, $38–53\,\mu$ (averaging 47) $\times 70–96\,\mu$ (averaging 85), thin-walled. Rhizoids numerous, $12–15\,\mu$ wide, smooth and whitish.   Underleaves distant, in one row on each side of the midrib, ovate, dentate.   Leaf-auricles generally two at the base of each lobe, oval, hollow, becoming filled with *Nostoc*-colonies and appearing as black dots through the thallus.   Involucre fusiform with a constricted mamillate apex, including tender and hyaline calyptra.   Capsule oval, pale olive brown, 1.2 mm. broad, 1.5–1.8 mm. long, with a collar at the base, dehiscing by 4 valves; capsule-wall consisting of 3–4 layers of cells, those of the outer layer with nodulose thickenings in radial walls; those of the inner layers smaller and tender without any thickenings; pedicel hyaline, with tint of pale green, 0.6–0.7 mm. broad, 15 30 mm. long.   Spores $35–61\,\mu$ in diameter, rounded-tetrahedral, yellowish-green, minutely papillose.   Elaters pale green, $13–16\,\mu$ broad, $236–321\,\mu$ long, with 2–4 spires. Elater-bearers rudimentary, at the bottom of the capsule. ♂ plant smaller; antheridia oval with a short pedicel, immersed singly in small alveoli of the dorsal side of the midrib. Gemmae of two kinds; one being spherical to oval, $92–99 \times 118\ 138\,\mu$ yellowish-green, multi-cellular and longly pedicellate, in flask-shaped receptacles with
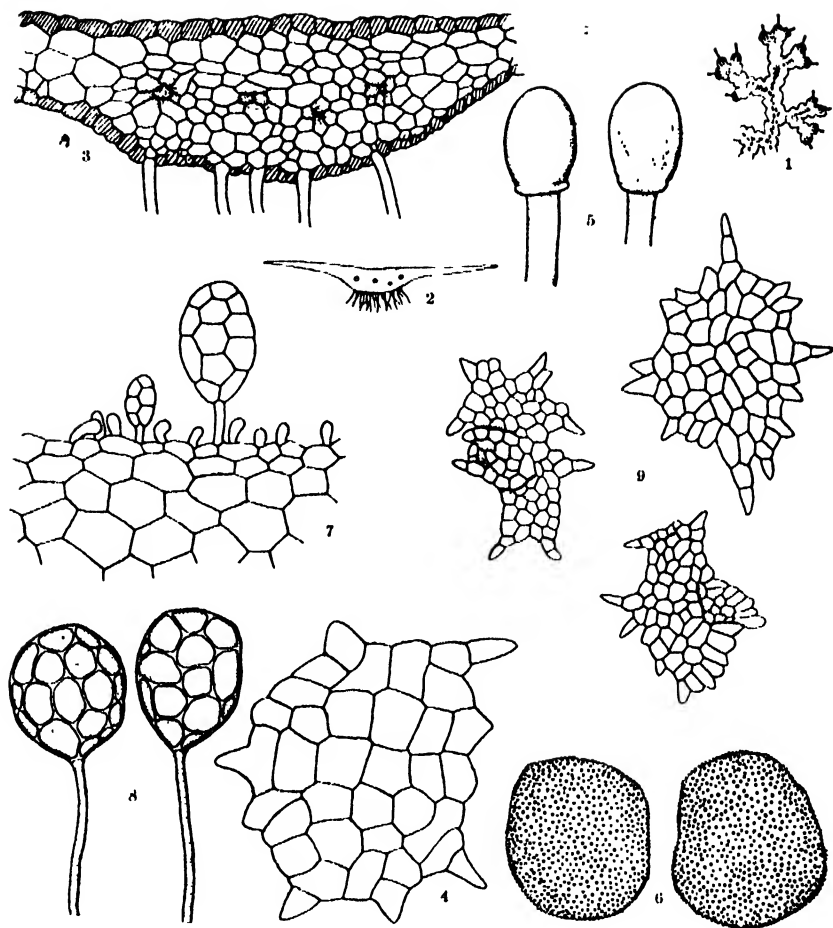
Text-Fig. 7. *Blasia pusilla* LINNÉ

1. ♂ thallus with antheridia, flask-shaped receptacles and stellate gemmae, in natural size. 2. Cross-section of thallus, ×12. 3. Middle part of ditto, ×75. 4. Underleaf, ×144. 5. Capsules, ×8. 6. Spores, ×490. 7. A part of basal portion of flask-shaped cupules, ×144. 8. Mature gemmae from ditto, ×144. 9. Stellate gemmae, ×75, the lower two under germination.

long necks situated on the dorsal side of the midrib; the other occurring as loose stellate scales on the dorsal surface near the apices of the thallus.

Fr. April-May.

Hab. On moist ground and wet banks.

Loc.

Honshiu : Tsuta, prov. Rikuchû (Y. HORIKAWA, Sept. 1928) ; Yamanaka-daira, prov. Rikuzen (Y. HORIKAWA, no. 1346, Nov. 1928) ; Osawa-mura, prov. Rikuzen (M. SHIBATA, no. 1016, Oct. 1927) ; Sendai, prov. Rikuzen (Y. HORIKAWA, no. 224, Sept. 1926, no. 263, March 1927 & S. OKABE, no. 1007, Sept. 1927) ; Katsura-shima, prov. Rikuzen (Y. HORIKAWA, no. 779, July 1927); Taira, prov. Iwaki (T. ONO, no. 1006, Aug. 1927); Mt. Temmoku, prov. Kai (K. TAMURA, no. 70, Oct. 1902) ; Mt. Komagatake, prov. Shinano (Y. HORIKAWA, no. 129, July 1923); Togo-mura, prov. Aki (I. KASHIMURA, no. 23, Oct. 1925) ; Mt. Fukuoji, prov. Aki (A. NOGUCHI, no. 3, Oct. 1926) ; Kure, prov. Aki (A. NOGUCHI, no. 4, Feb. 1928).

Text-Fig. 8. A marginal part of thallus of *Blasia pusilla* L., showing the state of succubous lobes, ×50.

Shikoku : Tochû, Hongawa-mura, prov. Tosa (T. YOSHINAGA, no. 1, Nov. 1928).

Kiushiu : Nankwan, prov. Higo (Y. HORIKAWA, no. 139, Sept. 1923., no. 200, Apr. 1924) ; Sakaki-mura, prov. Higo (Y. HORIKAWA,

no. 40, March 1923 & no. 314, Apr. 1927); Aida, prov. Higo (K. MAYEBARA, no. 8, Feb. 1927); Kagoshima, prov. Satsuma (Y. HORI-KAWA, no. 589 & 590, Apr. 1927).

Distrib. Europe, North America and Asia.

Genus: CAVICULARIA STEPHANI (1897)

Cavicularia densa STEPHANI
(Text-Figs. 9, 11, & Tables 1 2)

Cavicularia densa STEPHANI, Bull. Herb. Boiss. Vol. V, p. 87 (1897); MIYAKE, Bot. Magaz. Tokyo, Vol. 12, p. 85 (1898); SCHIFFNER, Oesterr. Bot. Zeitsch. Bd. 49, p. 391 (1899); HORIKAWA, Sci. Rep. Tôhoku Imp. Univ. 4th Series, Vol. III, p. 259 (1928).

Dioicous. Mesophyte or hygrophyte. Growing in deep green, densely caespitose patches. Thallus large, 4-10 mm. broad, and 2-5 cm. long, repeatedly dichotomous, the margin irregularly dentate. Midrib broader, the epidermal cells far smaller than the inner cells, 12-16 cells thick in the median portion, usually with 4-5 longitudinal bundles containing a deposit of $CaCO_3$, gradually passing into the 1-celled lobes. Lobes succubous, imbricate, semi-circular in form, 0.34-0.47×0.6-1.3 mm., the margin irregularly incised and somewhat undulating. Cells of lobes 5-8-angled, 23-34×23-57μ. Rhizoids numerous, 11-55 μ wide, smooth and whitish. Underleaves distant, in one row on each side of the midrib, ovate, dentate. Leaf-auricles generally two at the base of each lobe, oval becoming filled with *Nostoc*-colonies and appearing to the naked eye as black dots through the thallus. Involucre and calyptra resembling that of *Blasia pusilla* but larger. Capsule oval, brownish yellow, 1-1.7 mm. broad, 1.6-2.2 mm. long, with a collar at the base, dehiscing by 4 valves; capsule-wall consisting of 3 layers of cells, those of the outer layer with nodulose thickenings in radial walls; those of the inner layers tender, without any thicken-ings; pedicel hyaline with tint of pale green, 0.8-1 mm. broad, 26-55 mm. long. Spores 46-88 μ (mostly 62 μ) in diameter, nearly spheri-cal, greenish-yellow, minutely papillose. Elaters brownish yellow, 13-26 μ in breadth, to 472 μ in length, with 2-4 spires. Elater-bearers rudimentary, at the bottom of the capsule. ♂ plant smaller; antheridia oval with a short pedicel, immersed singly in small alveoli of the dorsal side of the midrib. Gemmae numerous in crescent-shaped
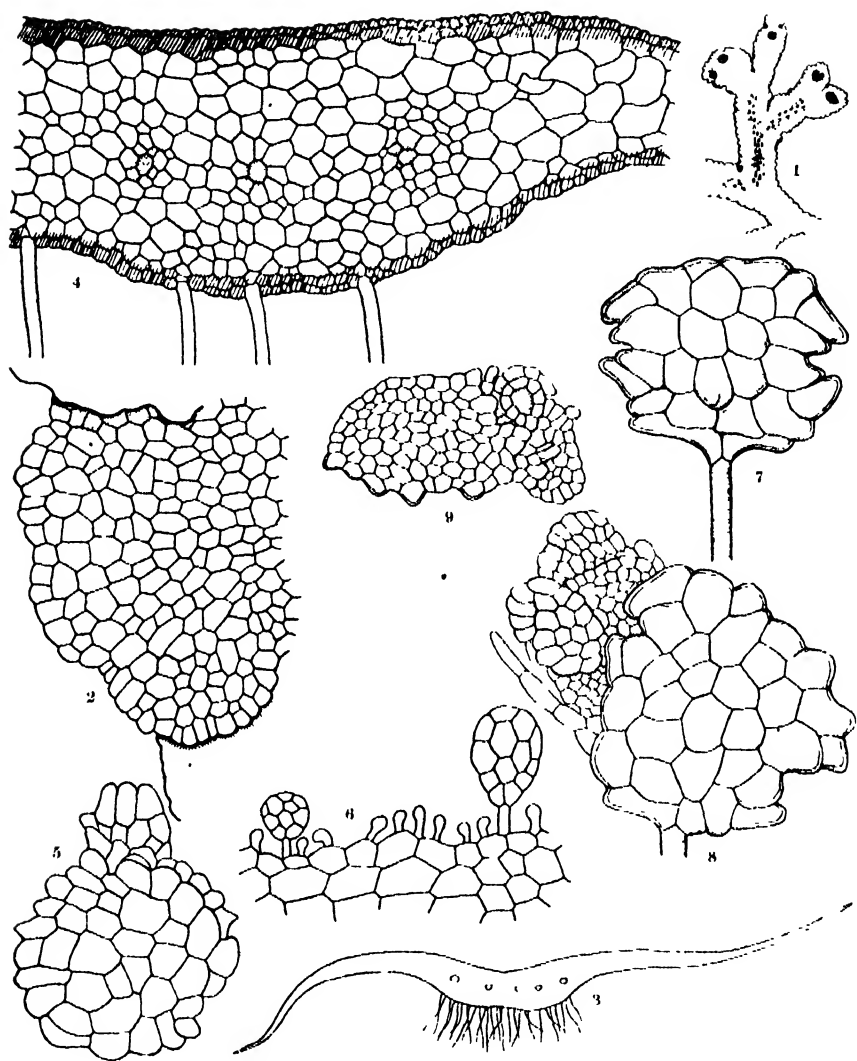
Text-Fig. 9. *Cavicularia densa* STEPHANI

1. Thallus with antheridia and crescent-shaped gemmae-receptacles in natural size. 2. Marginal portion of thallus, ×144. 3. Cross-section of thallus, ×12. 4. Half-part of middle portion of ditto, ×75. 5. Underleaf, ×144. 6. A part of basal portion of gemmae-cupules, ×144. 7. Mature resting gemma, ×144. 8. Ditto, under germination, ×144. 9. Younger gemma, under germination, ×144.

cupules situated on the dorsal side of the midrib, vertically inserted, with a long 1-celled hyaline pedicel, when mature brown and somewhat lenticular in form, 184–210 × 184–197 μ in diameter.

Fr. April-May.

Hab. On moist and wet banks in the mountainous regions.

Loc.

Honshiu: Tsuta, prov. Rikuchû (Y. HORIKAWA, no. 1278, Sept. 1928); Hanamaki, prov. Rikuchû (Y. HORIKAWA, no. 1183, May 1928); Hiraizumi prov. Rikuchû (Y. HORIKAWA, no. 1188, May 1928); Osawa-mura, prov. Rikuzen (M. SHIBATA, no. 1017, Oct. 1927); Sendai, prov. Rikuzen (E. IISHIBA, Oct. 1927); Tatsunokuchi, a vicinity of Sendai, prov. Rikuzen (Y. HORIKAWA, no. 225, Sept. 1926 & no. 262, March 1927, & no. 731, Apr. 1927); Kamakura, prov. Sagami (K. HISAUCHI, in Herb. Imp. Univ. Tokyo, no. 4, Jan. 1915); Kyoto, prov. Yamashiro (M. SHINMACHI, Oct. 1928).

Kiushiu: Ôhara-mura, Tamana-gôri, prov. Higo (Y. HORIKAWA, no. 317, Apr. 1927).

According to STEPHANI (1897), " Akita, Kamiiso, Towada, Tsurugi-zan. FAURIE 14862, 14034, 14499 ".

Distrib. The genus and species endemic.

Remarks on *Blasia* and *Cavicularia*.

In my preceding paper (1928) I have pointed out that *Blasia* and *Cavicularia* are very closely allied genera mainly with respect to the sporophyte. Some of the most important differences in the gametophyte are as follows:

		Blasia	*Cavicularia*
Thallus	....	annual & smaller	perennial & larger
		yellowish green, often tinged with purple	deep green, never tinged with purple
		flexuous	brittle
Lobes	....	more or less distant	imbricate
		larger, average 1.18 × 1.84 mm.	smaller, average 0.4 × 0.95 mm.
Lobe-cells....		47 × 85 μ	29 × 40 μ
Cupules.................		flask-shaped	crescent-shaped
Gemmae..............		two kinds	one kind

Concerning the gemmae of *Cavicularia*, one controversy exists

among the writers. SCHIFFNER[1] and GOEBEL[2] maintain that there are
two sorts of gemmae in the cupules of this plant and STEPHANI[3]
recognizes only one kind of gemmae.   After examination of numerous
materials the author was able to come to the conclusion that the
gemmae of *Cavicularia* are when mature typically of a definite, longly
pedicellate, thick-walled form (*Dauerbrutkörper*), but some gemmae

TABLE   1.

Sexual dimorphism of *Blasia pusilla* and *Cavicularia densa*
Width of thalli in ♀ & ♂.

Width groups in mm.	*Blasia pusilla* ♀ Frequency (f)	Sum of group-values	Mean in each group	*Blasia pusilla* ♂ Frequency (f)	Sum of group-values	Mean in each group	*Cavicularia densa* ♀ Frequency (f)	Sum of group-values	Mean in each group	*Cavicularia densa* ♂ Frequency (f)	Sum of group-values	Mean in each group
0.5– 0.8				4	32.0	0.8						
0.9– 1.2				52	59.2	1.1_4						
1.3– 1.6				142	217.4	1.5_4						
1.7– 2.0				104	193.8	1.8_0				1	2.0	2.0
2.1– 2.4	3	7.0	2.3_5	79	178.8	2.2_6				11	25.6	2.3_5
2.5– 2.8	16	43.4	2.7_1	62	165.2	2.2_6				40	108.6	2.7_2
2.9– 3.2	37	116.0	3.1_4	41	125.8	3.0_9				61	189.6	3.1_1
3.3– 3.7	73	255.8	3.5	16	55.0	3.4_4				73	255.6	3.5
3.8– 4.1	117	457.6	3.9_1							84	328.4	3.9_1
4.2– 4.5	111	477.4	4.3							85	366.8	4.3_2
4.6– 4.9	77	361.4	4.6_9							60	282.0	4.6
5.0– 5.3	40	203.4	5.0_9				4	20.6	5.1_5	44	223.4	5.0_8
5.4– 5.7	18	98.4	5.4_7				17	94.2	5.5_4	26	142.2	5.4_7
5.8– 6.1	5	29.2	5.8_4				30	177.6	5.9_2	14	82.2	5.8_7
6.2– 6.5	3	18.8	6.2_7				41	258.6	6.4_7	1	6.2	6.2
6.6– 6.9							65	435.8	6.7			
7.0– 7.3							76	540.2	7.1_1			
7.4– 7.7							97	727.2	7.5			
7.8– 8.1							67	528.6	7.8			
8.2– 8.5							46	381.4	8.2_0			
8.6– 8.9							25	217.4	8.7			
9.0– 9.3							22	200.2	9.1			
9.4– 9.7							9	85.2	9.4_7			
9.8–10.1							1	9.8	9.8			
Total sum	500	2068.4		500	1027.2		500	3676.8		500	2012.6	

[1] SHIFFNER, V., Oesterr. Bot. Zeitsch., p. 392 (1899).
[2] GOEBEL, K., Organographie d. Pflanzen, Zweiter Teil, p. 670 (1915–18)
[3] STEPHANI, F., Species Hepaticarum, Vol. I, p. 363 (1900).

TABLE 2.

Sexual dimorphism of *Blasia pusilla* and *Cavicularia densa*.
Measure of mean (M) and standard deviation (σ) in mm.

		Mean (M) P. E mean	M♀ — M♂ P. E diff. mean (a, b)	Standard deviation (σ) P. E σ	Coefficient of variation (v) P. E v
Blasia pusilla	♀	$4.13_4 \pm 0.02_1$	$2.12_8 \pm 0.02_5$	$0.70_2 \pm 0.01_4$	$16.98_6 \pm 0.57_0$
	♂	$2.00_6 \pm 0.01_9$		$0.65_1 \pm 0.01_1$	$27.52_1 \pm 0.61_4$
Cavicularia densa	♀	$7.?5_2 \pm 0.02_8$	$4.32_0 \pm 0.03_8$	$0.94_2 \pm 0.02_0$	$12.81_6 \pm 0.28_2$
	♂	$3.02_2 \pm 0.02_0$		$0.86_7 \pm 0.01_8$	$28.71_1 \pm 0.65_7$

being already germinated when young within the receptacle, appear to be very divergent from the mature ones. Yet as all the kinds of transitional forms between these two extremes are found, and as all of them are no doubt of a homologous origin as already discussed by GOEBEL, it will be most reasonable to regard the gemmae of *Cavicularia* to be in reality of only one kind.

Blasia and *Cavicularia* are both monotypic genera and are strictly dioicous. Since some years ago I have noticed the fact that in these plants the female thalli are far larger than the male. Fortunately they are growing very abundantly in the vicinity of Sendai. I estimated, in the middle of August 1927, the width of ♀ and ♂ thalli with " Messlupe " (unit in 0.2 mm.). A thousand individuals of *Blasia* (♀ 500, ♂ 500) and the same numbers of *Cavicularia* (♀ 500, ♂ 500) were used for the estimation. The results are given in Table 1-2 and Text-Figs. 10 11.

Recently LORBEER[1] reported that *Pellia Fabbroniana* and *P. Neesiana* represent a distinct sexual dimorphism but he does not recognize it in *Blasia pusilla*. Over ten years ago LORCH[2] described " Männl. Pflanzen kleiner ". At any rate, so far as the author is concerned, Japanese specimens of *Blasia pusilla* are representatively good examples of the sexual dimorphism, as well as *Cavicularia densa*.

---

[1] LORBEER, G., Zeitsch. f. Induk. Abstamm. u. Vererb., Bd. XXXXIV, p. 24 (1927).
[2] LORCH, W., Die Torf- u Lebermoose, in LINDAUS Kryptogamenflora f. Anfänger, p. 86 (1914).
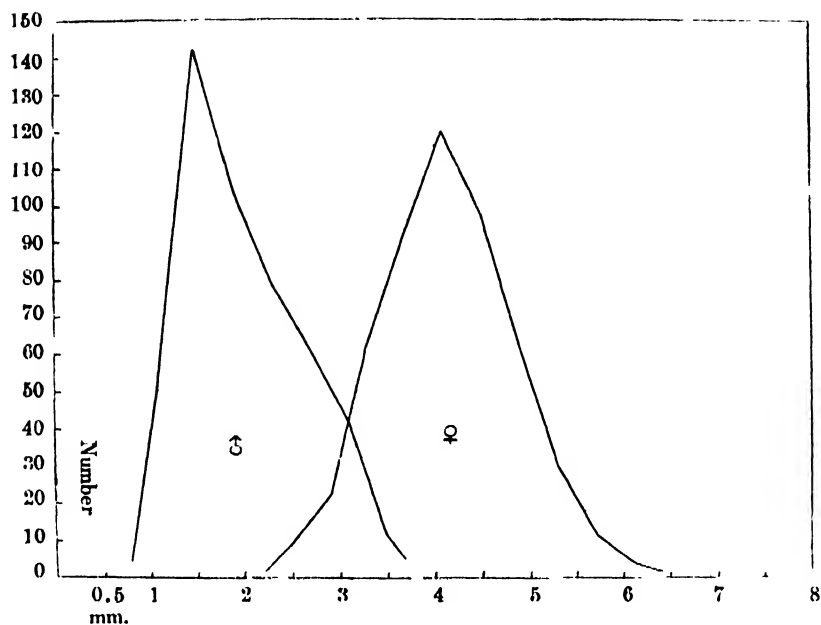
Text-Fig. 10. Diagrammatic polygons, showing the width of ♂ & ♀ thalli in *Blasia pusilla*.
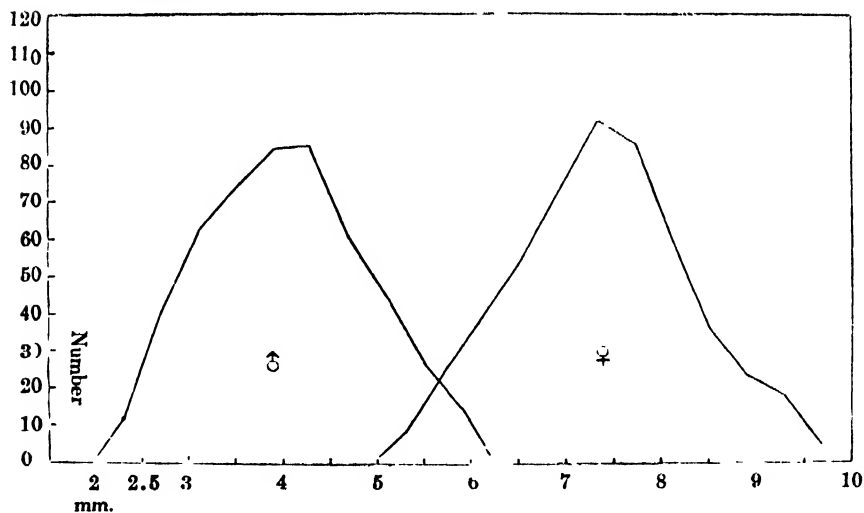
Text-Fig. 11. Diagrammatic polygons, showing the width of ♂ & ♀ thalli in *Cavicularia densa*.

Genus: CALOBRYUM Nees (1836)

Syn. *Rhopalanthus* Lindberg (1874)

Scalia Spruce (1885)

Calobryum rotundifolium (Mitt.) Schiffner
(Pl. XVII.)

Rhopalanthus mnioides Lindberg, Manip. Musc. II, p. 390 (1874) & Hedwigia, p. 139 (1875).

Scalia rotundifolia Mitten, Trans. Linn. Soc. London, 2nd Series, Vol. III, p. 204 (1891).

Calobryum rotundifolium (Mitt.) Schiffner, Oesterr. Bot. Zeitschr., p. 389 (1899); Ikeno, Shokubutsu Keitô-gaku, Vol. II, p. 354, Fig. 223 (1922).

Calobryum mnioides (Lindb.) Stephani, Spec. Hepat. Vol. I, p. 399 (1900); Okamura, in Makino's Nipp. Shokub. Dzukwan, p. 1260 (1925); Hayata, Bot. Magaz. Tokyo, Vol. XLII, p. 183 (1928).

Calobryum rotundifolium (Mitt.) Stephani, Spec. Hepat. Vol. VI, p. 76 (1917).

Dioicous. Mesophyte. Plants growing in oblique or erect loose tufts, usually 1.5–2.3 cm. long and 5–6 mm. wide, pale green when fresh, dark green and shrinking when dry. Stems 1 mm. in diameter, with the pale yellowish basal rhizomatous portion, simple, thick and succulent, in sterile one equal-sized everywhere, in fertile larger at upper part, in cross-section slightly compressed dorsiventrally, therefore broad elliptical, usually about 20 cells wide, cells large and thin-walled; in the centre there exists a small-celled conducting bundle. Rhizoids absent. Leaves entire, suborbicular, smaller below and larger above, arranged distinctly in three rows; in sterile and ♀ in two rows, lateral leaves equal-sized, approximate, alternate, patent, suborbicular in outline, dorsal leaves (according to Stephani, *amphigastria*) in one row, smaller and alternate with the lateral leaves, approximate; in ♂ stem three rows of leaves similar in size, distant, uppermost three leaves usually largest. Leaf-cells plane and 4–7-angled, 27.5–57.7 μ at the apex, 27.5–55 × 55–96.3 μ in the middle and 35.7–55 × 94–110 μ at the base, walls thin and angles not thickened, with numerous chlorophyll granules. Perianth absent. Calyptra much-exserted from the stem leaves, linear-oblong, nearly hyaline, 7–9 mm. in length and 1.4 mm. in diameter; the wall consisting of a 5–6-celled layer. Capsule cylindrical, brown, 3.5 mm. in length and 0.93 mm. in diameter, dehi-

scing to the base usually on one longitudinal line. Capsule-wall consisting of a single one-celled layer, each cell with a brownish-yellow annular thickening. Pedicel 3 cm. in length, hyaline, hollow, wall 4-5 cell-thick. Spores spherical, 22-25 μ in diameter, pale yellow and minutely papilose. Elaters very long, usually 590-740 μ long and 5.5-7 μ broad, pale yellow, bispiral and loosely twisted, longly attenuate to slender extremities. Archegonia and antheridia numerous on each terminal receptacle.

Fr.   April-May.

Hab.   In moist ground and among other mosses in forests.

Loc.

Honshiu :   Sendai, prov. Rikuzen (Y. HORIKAWA, no. 229, Sept. 1926) ; Karasuzawa, Aomi-zinsha, Kamo-machi, prov. Echigo (B. HAYATA, 1894) ; Hiroshima, prov. Aki (Y. HORIKAWA, no. 63, Apr.— May 1923) ; Insl. Miyajima, prov. Aki (Y. HORIKAWA, no. 651, Apr. 1927 & A. NOGUCHI, Feb. 1929).

Shikoku :   Ogawa-mura, Takaoka-gôri, prov. Tosa (T. YOSHINAGA, 1899).

Kiushiu :   Mt. Aoi-dake, prov. Hiuga (Y. HORIKAWA, no. 448 & no. 476, Apr. 1927) ; Aoshima-mura, prov. Hiuga (Y. HORIKAWA, no. 394, Apr. 1927) ; Kagoshima, prov. Satsuma (Y. HORIKAWA, no. 550, Apr. 1927).

Distrib.   The species endemic.

Genus : PTILIDIUM NEES (1833)

Ptilidium pulcherrimum (WEB.) HAMPE
(Pl. XVIII, Figs. 10-15 & Text-Fig. 12)

Jungermannia pulcherrima WEBER, Specilegium Fl. Gottingensis, p. 150 (1836)

Ptilidium pulcherrimum HAMPE, Prod. Fl. Hercyn., p. 76 (1836).

Blepharozia pulcherrima LINDBERG, Musci Scand., p. 5 (1876).

Ptilidium ciliare (L.) HAMPE, var. *pulcherrimum* WARNSTORF, Krypt. Fl. Mark Brand., p. 26 (1902).

Dioicous. Xerophyte. In loosely scattered yellowish-green tufts. Plant attaining to 1.2–1.3 mm. in width and 4–4.5 cm. in length. Stems 0.2–0.24 mm. in diameter, rather firmly attached to the substratum, yellowish-green, prostrate, irregularly branched, branches

shortly pinnate and frequently bipinnate. Rhizoids colourless at the base of the underleaves. Leaves 1 mm. long and 0.48 mm. broad, approximate below, imbricate above and on the branches, semi-antical portion larger, 8-11 cells broad at the base, patent or erect-patent and divided near to the middle into two unequal triangular lanceolate segments, the margins with 10-13 long, rigid and one-cell rowed cilia;

Text-Fig. 12. *Ptilidium
pulcherrimum* (WEB.) HAMPE
1. Leaf, dissected from the
stem, ×38   2. Cilium from the
antical margin of a leaf, ×233.

the postical portion smaller, patent, deeply divided into two lanceolate segments with 5 6 longer curved or straight cilia. Leaf-cells 37 μ in the middle, 25 36 × 36-51 μ at the base, roundish oblong, the trigones large with often intermediate thickenings. Underleaves of stem somewhat distant, about half as large as the leaves, 578 μ wide, 525 μ long, appressed, rotund-quadrate, ventricose, the upper margins densely ciliate, the cilia frequently furcate lower half entire. Underleaves of the branches smaller than the stem-underleaves, contiguous to loosely imbricated, 263 μ wide, 237 μ long, more ventricose. -♀- inflorescence terminal on the stem or main branches, being later on short lateral branches through innovations. Involucral bracts embracing the perianth, erect, resembling the leaves, the margins densely ciliate. Perianth 3-3.5 mm. long and 1 -1.2 mm. wide, of one-cell layer, large, longly exserted, oblong-cylindrical, smooth, gradually narrowed towards the base, obtusely plicate above, the mouth contracted and 4-lobed and ciliate.

Fr.   July-August.

Hab.   On the bark of trees in the alpine districts.

Loc.

Honshiu :   Mt. Hakkôda, 1300 m., prov. Mutsu (Y. HORIKAWA, no. 1288, Sept. 1928) ; Mt. Iwate, 1400 m., prov. Rikuchû (S. HABARA,

no. 1272, July 1928).

Distrib. Europe, North America, Siberia, Canada and Alasca.

Genus: **LOPHOLEJEUNEA** Spruce (1884)

Key to the Japanese species

Stems rigid; leaves very closely imbricate; underleaves closely imbricate and 2-2.5 times broader than the stem ... *L. densiloba*

Stems flexuous; leaves contiguous to loosely imbricate; under-leaves distant and 5-6 times broader than the stem . *L. javanica*

Lopholejeunea densiloba HORIKAWA, sp. nov.
(Text-Fig. 13.)

Dioica. Planta caespitosa, brunneo-virens, corticola, 2-3 cm. longa et 2.5 mm. lata. Caulis prostratus, 0.23 mm. in diam., irregulariter pinnatus. Folia caulina dense imbricata, patula, parum convexa, oblongo-ovato, falcata, 1.46 mm. longa, 1.15 mm. lata, margine antico arcuato caulem valde superante, postico parum curvato, apice vulgo obtusissima. Cellulae basi 30-38 × 36-47 μ diam., medio 28-38 μ diam., margine 17-23 μ diam., trigonis majusculis, parietibus validis. Lobulus ovatus, 0.35 mm. longus, 0.3 mm. latus, inflatus, dente apicali introrso (angulo 25°), 6 cell. longo, basi 4 cell. lato. Amphigastria caulina magna, dense imbricata, reniformia, caule 5-6-plo latiora (0.7 mm. longa, 1.3 mm. lata) apice late truncata, basi abrupte acuta et decurrentia. Androecia in ramis terminalia, bracteis 6-12 jugis.

Hab. On the bark of trees.

Loc. Caroline Is., Palau I. (H. SATÔ, no. 1271, June 1928).

Dioicous. Xerophyte. Plants growing in depressed brownish green patches, 2-3 cm. long and 2.5 mm. broad. Stems prostrate, very rigid, 0.23 mm. in diameter, irregularly pinnate, the branches widely spreading, simple, usually with smaller leaves than the stem. Leaves closely imbricate, the lobe somewhat convex, widely spreading, falcate, oblong-ovate, averaging 1.46 mm. long, 1.15 mm. wide, antical margin arching far beyond the axis, postical margin slightly curved, forming an obtuse angle with the keel, apex very obtuse, margin entire throughout Cells of lobe nearly plane, averaging 30-38 × 36-47 μ at the base, 28-38 μ in the middle and 17-23 μ at the margin, middle lamella

distinct, trigones large, intermediate thickenings occasional, oval in out-
line, free walls of cells uniformly thickened.   Lobule ovate in outline,
averaging 0.35 mm. long, 0.3 mm. wide, inflated throughout, forming
an angle of about 108° with axis (and base of lobule), more or less
decurrent, keel strongly arched, free margin involute near the base,
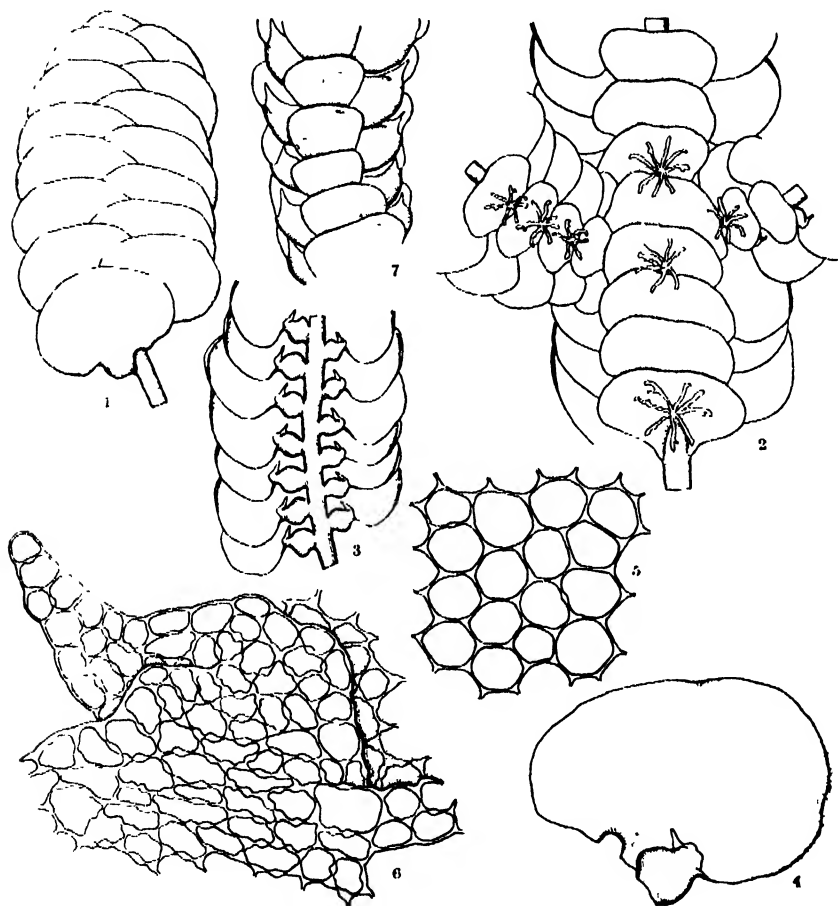
Text-Fig. 13. *Lopholejeunea densiloba* HORIKAWA
    1. Apex of plant, antical view, ×12.   2. Part of plant with two bases of
branches, postical view, ×12. 3. Part of stem, the underleaves dissected away
to show the lobules, postical view, ×12. 4. Stem-leaf, ×25. 5. Cells from middle
of lobe, ×233. 6. Apex of lobule, ×233. 7. A part of androecium, ×25.

sinus broad and adnate portion two cells across, apical tooth long and forming an angle of 25° with the axis, usually six cells long and 4 cells wide at the base. Underleaves densely imbricated, 5–6 times broader than the stem, nearly plane, reniform, 0.7 mm. long, 1.3 mm. wide, convex, more or less revolute and broadly truncate at the apex, abruptly narrowed and very long-decurrent at the base. Androecium occupying a short, slender branch ; bracts in from six to twelve paris, subequally bifid, the lobule narrower and shorter than the lobe, strongly inflated, obliquely spreading, lobe rounded, lobule acute at the apex, keel arched ; bracteoles loosely imbricated, reniform but more elongated.

Distrib.   This species endemic.

Remarks.   The present species is alike to *L. asiatica* STEPH. and *L. inermis* STEPH., in the leaves and underleaves. But it differs from them in the characters found in the inflorescence, stem, androecium and lobule.

Lopholejeunea javanica (NEES) STEPHANI
(Text-Fig 14)

Lejeunea javanica NEES, Syn. Hepat., p 320 (1844)
Lopholejeunea javanica (NEES) STEPH, Spec Hepat, Vol V, p 84 (1912)

Monoicous. Xerophyte. Plants growing in depressed brown patches, 1.5–2.5 cm. long and 1.5 mm. broad. Stems prostrate, flexuous, 0.16 mm. in diameter, irregularly pinnate, the branches usually simple, often with smaller leaves than the stem. Leaves contiguous to loosely imbricate, the lobe slightly convex, widely spreading, somewhat falcate, ovate, 1 mm. long, 0.75 mm. wide, antical margin arching partially or slightly across the axis, more or less outwardly curved from the base to the apex, postica margin slightly curved, forming an obtuse angle with the keel, apex rounded to very obtuse, margin entire throughout. Lobule ovate in outline, 0.35 mm. long, 0.22 mm. wide, inflated throughout, slightly decurrent, forming an angle of about 70' with axis (and base of lobule), free margin slightly revolute to apex, sinus broad and shallow, adnate portion one cell across   Cells of lobe averaging 21 μ at the margin, 25 μ in the middle and 51×31 μ at the base, trigones distinct, triradiate with acute rays, intermediate thickenings occasional, oval. Underleaves distant, about twice (2–2.5) as broad as the stem,

plane or nearly so, rounded to reniform, 0.27 mm. long, 0.35 mm. wide,
rounded and slightly decurrent at the base, apex broad, rounded or
somewhat truncate, margin entire. Inflorescence terminal on a short
branch, with two or one innovations: bracts similar to the leaves,
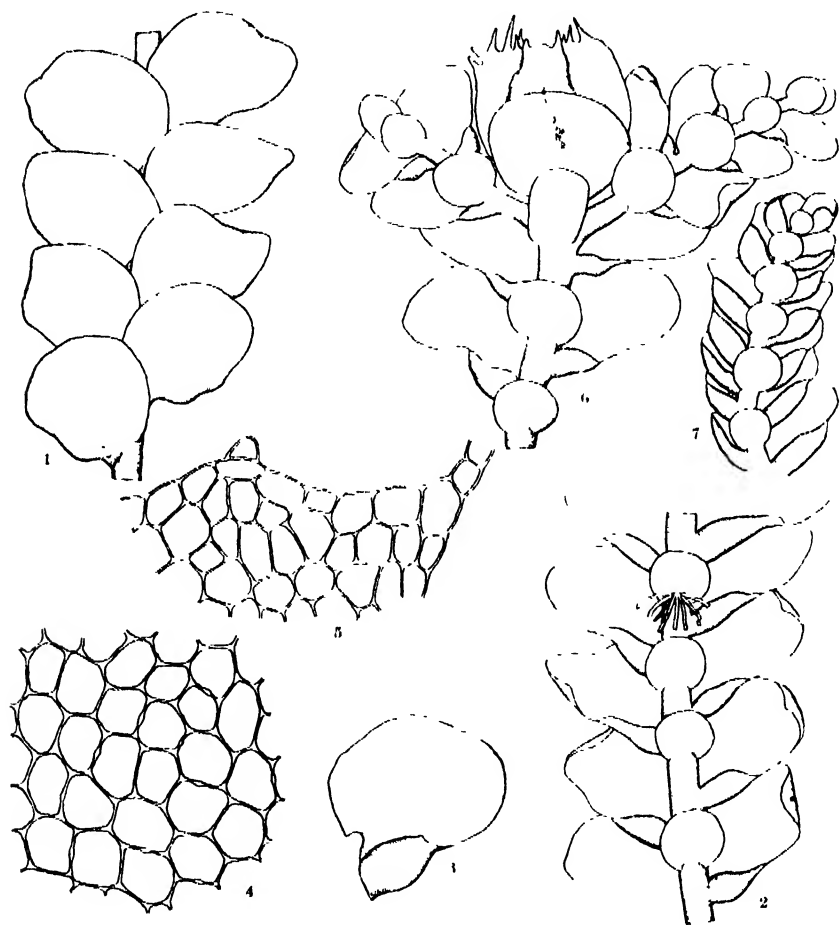
Text-Fig. 11. *Lopholejeunea javanica* (NEES) STEPHANI

1. A part of stem, antical view, ×25. 2. Ditto, postical view, ×25. 3. Stem-
leaf, ×23. 4. Cells from middle of lobe, ×233 5. Apex of lobule ×233. 6.
Part of plant with perianth, postical view, ×25 7. Androecium postical view,
×23.

bracteole free, broadly orbicular, 0.6 mm. long, 0.76 mm. wide, plane,
apex broadly rounded to truncate, margin quite entire. Perianth about
1/4 exserted, obovate in outline, 0.93 mm. long, 0.82 mm. wide, beak
short, mouth irregularly dentate, the teeth more than 1 cells long and
one or two cells wide at the base, surface otherwise smooth. Andro-
ecium occupying a short lateral branch: bracts in about six pairs,
strongly inflated, keel broadly arched, apex obtusely pointed, bracteoles
similar to the underleaves but smaller.

Loc.

Shikoku: Yasui, prov. Tosa (T. YOSHINAGA, no. 21, Sept. 1898).

Distrib. Asia, Tropical Oceania and Oriental Africa.

Genus: NOTOTHYLAS SULLIVANT (1846)

Notothylas japonica HORIKAWA, sp. nov.
(Pl XVIII, Figs 1-9.)

Monoica. Planta parva, saturate viridis, expansa vel substratificata,
terricola. Frons ad 10–15 mm. in diametro, orbicularis, lobis obovatis,
margine lobulis cuneatis grossisque instructis. Involucra solitaria,
cylindrica, attenuata, apice scariosa. Capsula valida, 4 mm. longa,
fusco brunnea, cylindrica. Columella nulla. Sporae 38 μ in diametro,
leves, nigrae. Pseudo-elateres 32–64 μ longi.

Fr. Sept. — Oct.

Hab. Moist soil in fields and gardens.

Loc.

Honshiu: Tsuta, prov. Rikuchû (Y. HORIKAWA, Sept. 1928);
Sendai, prov. Rikuzen (Y. HORIKAWA, no. 1340, Oct. 1928); Koishi-
kawa Bot. Garden, Tokyo (Y. HORIKAWA, Sept. 1928); Kabe, prov.
Aki (Y. HORIKAWA, Oct. 1925).

Monoicous. Mesophyte. Growing in deep green to brownish green
patches. Thallus 10–15 mm. in diameter, orbicular, divided into broad
obovate lobes, with the margin much irregularly divided into cuneate
lobules, the dorsal surface more or less warty, not costate; surface
cells 32–53 × 89–116 μ, each cell with a large chloroplast; cross-section
8–10 cells high in the middle, becoming 1 or 2 cells high at the
margin, the interior cells hyaline and much larger than the epidermal

cells, lacunae large and numerous. Involucres usually single, 2.1–
2.5 mm. long and 1-1.4 mm. broad, cylindrical, narrowed toward the
apex, the mouth scarious. Capsule valid, 3–4 mm. long and 0.6–0.8 mm.
broad, blackish-brown and cylindrical, the wall consisting of 3-5 cell-
layers; the outermost layer, one-cell (18 × 17 μ in cross-section) in
thickness, with thickened and brownish-yellow walls; the inner layer,
2-4 cells in thickness, with thin and hyaline walls. Columella absent.
Spores 37–39 μ in diameter, smooth and black. Pseudo-elasters 32–
64 μ long with yellowish spires. Antheridia 53 × 106 μ, oval, shortly
pedicellate.

Distrib. The species endemic.

Remarks. Up to the present day, eleven[*] species of *Notothylas*
have been recognized. Of these, the present new species is nearly
related to *N. orbicularis* (SCHWEIN.) SULL., but the former differs from
the latter in the size of capsule, spores, the state of thickenings of
capsule-walls, and the absence of columella. This is the first time
that the genus *Notothylas* is reported within our boundaries, but it
appears to be widely distributed in Japan.

Genus: ANTHOCEROS (L. 1753) ref. GOTTSCHE (1858)

Anthoceros gemmiferus HORIKAWA, sp. nov.
(Text-Fig 15.)

Sterilis. Planta caespitosa, fusco-virens, terricola. Frons repitito-
furcata, 1.5-2 cm. longa et 3-4 mm. lata, in sectione transversa solida
et tenax, medio 8-10 cellulas crassa, lobis ligulatis, subtus margineque
gemmiferis. Gemmae valde numerosae, magnae, ovales vel pyriformes,
multicellulares, virides.

Hab. More or less moist soil.

Loc.

Honshiu: Sendai, prov. Rikuzen (Y. HORIKAWA, no. 1349, Dec.
1928); Obara, Shiraishi-gôri, prov. Rikuzen (Y. HORIKAWA, no. 1021,
Oct. 1927).

Sterile. Mesophyte. Plants growing in dark green, extended
caespitose patches. Thallus repeatedly dichotomous, 1.5–2.0 cm. long

*STEPHANI. F., Spec. Hepat. Vol. V, p. 1019 (1917).

and 3-4 mm. broad, slightly convex above, divided into ligulate lobes. Cross-section 8-10 cells thick in the middle without lacunae. Surface cells $21-34 \times 40-53\,\mu$, each cell with a large chloroplast. Gemmae exceedingly numerous at the margin and the ventral surface, multicellular, oval to pyriform, green, $197-250 \times 211-276\,\mu$ in diameter.
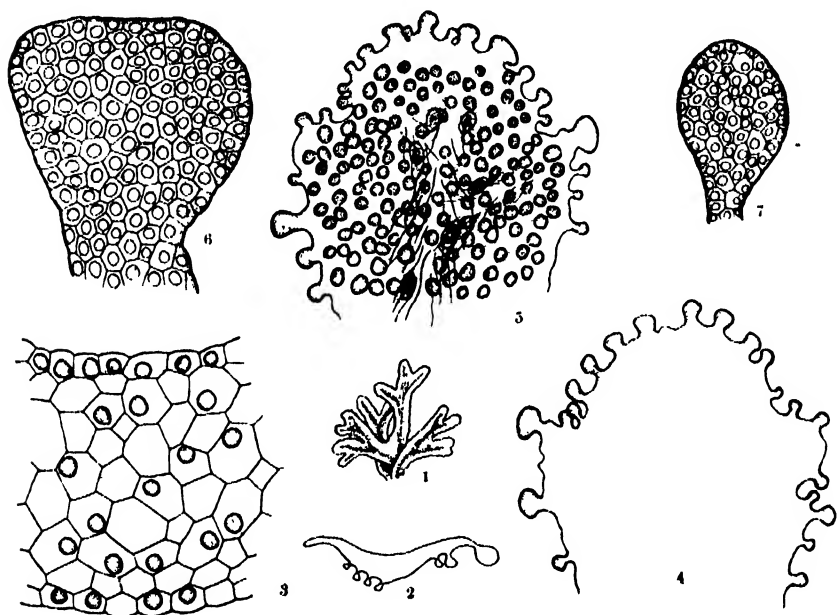
Text-Fig. 15. *Anthoceros gemmiferus* HORIKAWA

1. Habit of plants, in natural size. 2. Cross-section of thallus, × 12. 3. Middle part of ditto, × 144. 4. Anterior part of thallus, antical view, × 12. 5. Ditto, postical view, × 12. 6. Gemma from the margin of thallus, × 144. 7 Gemma from the ventral surface of thallus, × 144.

Remarks. According to STEPHANI's description*, among many species of the genus, the present species seems most closely allied to *Anthoceros propaguliferus* STEPH., which is known only from Concepcion of Chile.

---

*STEPHANI, F.. Spec. Hepat. Vol. V, p. 1001 (1916).

EXPLANATION OF PLATES.

PLATE XVI.

Fimbriaria Yoshinagana HORIKAWA

Fig. 1. Anterior part of thallus with the stalk of ♀ receptacle, ×8.
Fig. 2. ♀ receptacles, ventral view, ×8.
Fig. 3 Cross-section of thallus, middle portion, ×144.
Fig. 4 Epidermal pore of thallus, ×233.
Fig. 5. Epidermis, one cell containing a oil-body, ×233.
Fig. 6. Cross-section of the stalk of ♀ receptacle, ×75.
Fig. 7. A lobule of perianth, ×23.
Fig. 8. Cells from ditto, ×144.
Fig. 9 Capsule-wall, surface-view, ×144.
Fig. 10. Spore, ×460
Fig. 11 Elater, ×460.
Fig. 12. Branching elater, ×460

PLATE XVII

Calobryum notundifolium (MITT) SCHIFFNER

Fig. 1 ♀ plant bearing mature sporophyte, ×2.
Fig 2. Upper part of stem with younger sporophyte, ×2
Fig. 3 ♂ plant bearing antheridial receptacle, ×2.
Fig 4. Cross-section of stem, ×12
Fig 5 A part of ditto, ×75.
Fig. 6. A leaf, dissected from the stem, ×12
Fig. 7 Cells from the middle part of lobe, ×144
Fig 8 Cells from the marginal part of lobe, ×144.
Fig 9. Cross-section of calyptra, middle portion, ×12.
Fig. 10 A part of ditto, ×144.
Fig 11. Cross-section of pedicel, ×12.
Fig. 12 A part of ditto, ×144.
Fig. 13. Dehiscent capsule, ×8.
Fig. 14. Capsule-wall, surface view, ×233.
Fig. 15. Cross-section of capsule-wall, ×233.
Fig 16. Longitudinal-section of capsule-wall, ×233.
Fig 17. Spore, ×460
Fig. 18. Elater, ×233.

PLATE XVIII.

Notothylas japonica HORIKAWA

Fig. 1. Growing-habit of plant bearing sporophytes, in nat. size.

Fig. 2.   A part of ditto. ×8.

Fig. 3.   Nearly mature capsule, ×12.

Fig. 4.   Outer-layer of cells of ditto, surface view, ×233.

Fig. 5.   Cross-section of capsule-wall, ×460.

Fig. 6.   Longitudinal-section of ditto, ×233.

Fig. 7.   Spores, ×300.

Fig. 8.   Elaters, ×300.

Fig. 9.   Antheridium, ×300.

Ptilidium pulcherrimum (WEB.) HAMPE

Fig. 10.   A part of plant, antical view, ×38.

Fig. 11.   A part of plant, postical view, ×38.

Fig. 12.   Cross-section of stem, ×233.

Fig. 13.   Cells from the middle part of lobe, ×233.

Fig. 14.   Perianth, ×16.

Fig. 15.   Cross-section of perianth, above the middle, ×23.
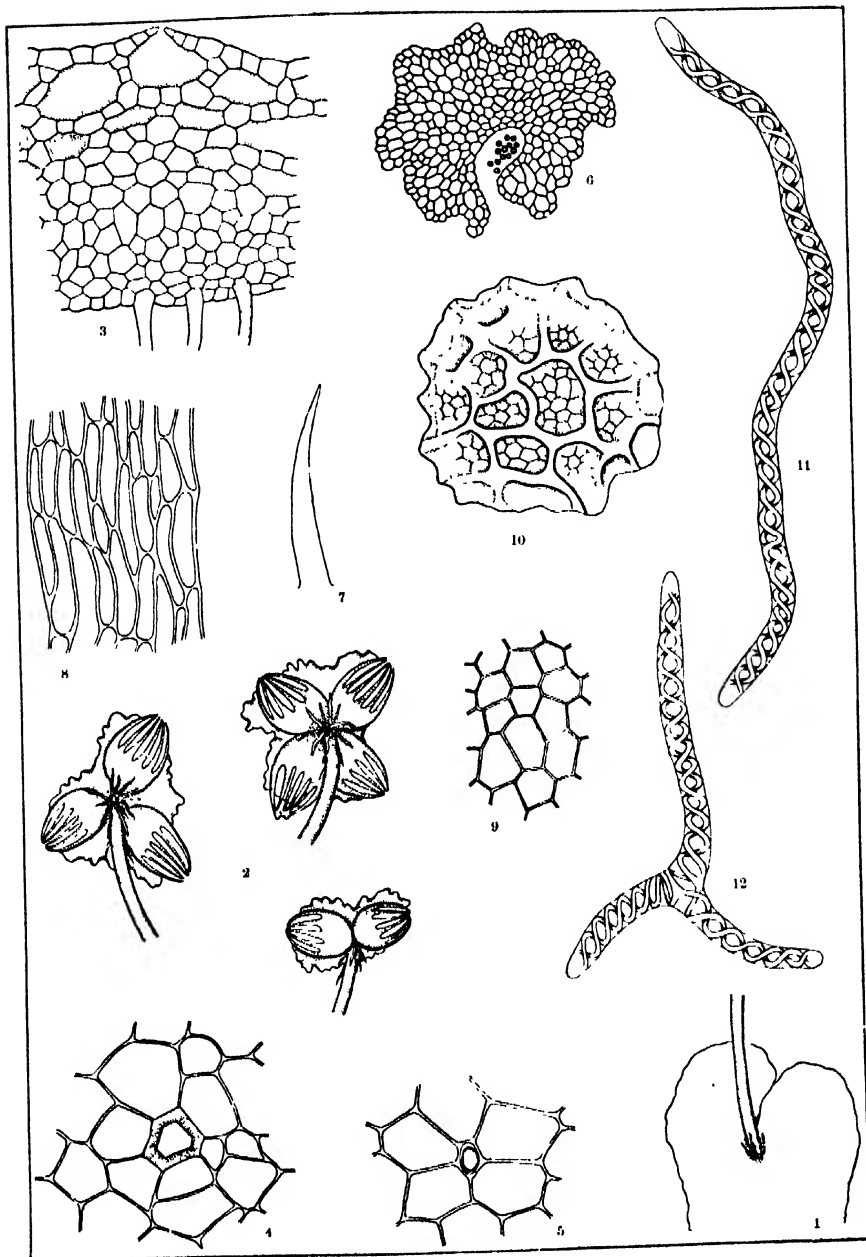
Y. Horikawa: *Fimbriaria Yoshinagana* sp. nov.
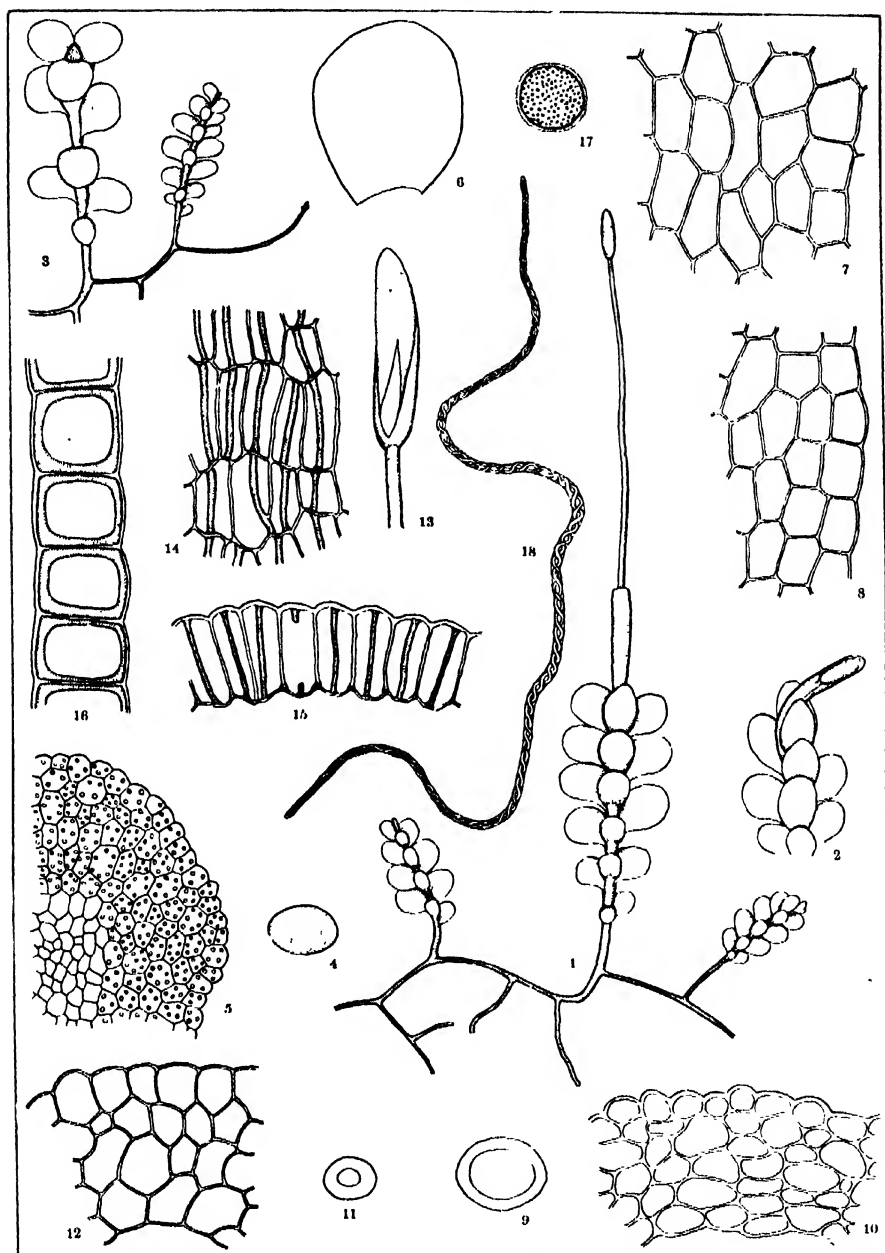
Author del.

Y. HORIKAWA: *Calobryum rotundifolium* (MITT.) SCHIFFNER.
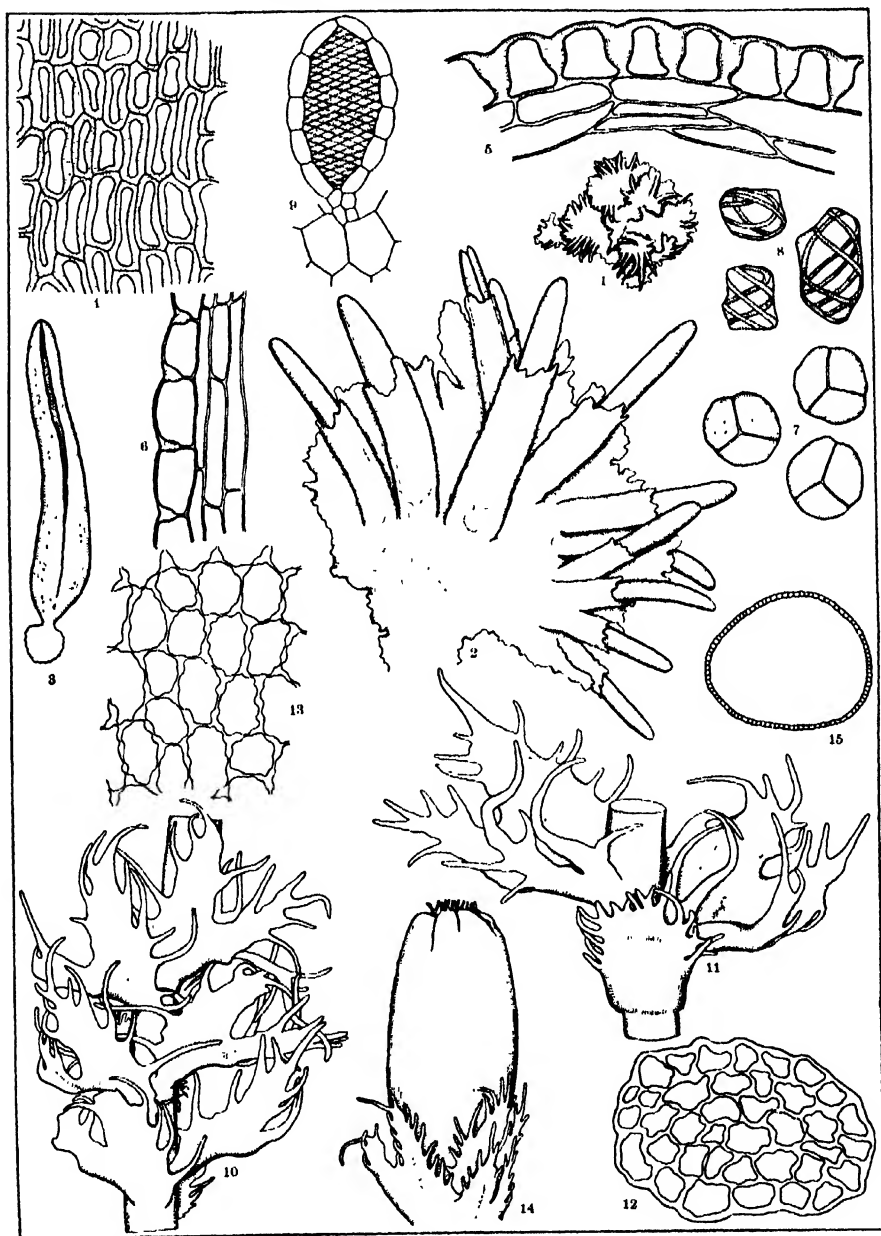
Y. HORIKAWA : *Notothylas japonica* sp. nov. & *Ptilidium*
pulcherriumum (WEV.) HAMPE.

Über die Wirkung der Elektrolyten auf den Sauerstoffverbrauch von *Chlorella ellipsoidea*.

Von

MANNEN SHIBATA.

(Biologisches Institut der Kaiserlichen Tôhoku Universität, Sendai.)

I. EINLEITUNG.

Als allgemeine Energiequelle der Pflanzen steht die Atmung mit zahlreichen anderen Lebenserscheinungen in inniger Beziehung, und von ihr sind die anderen physiologischen Vorgänge der Pflanzen mehr oder weniger abhängig. Es ist daher nicht nur von Bedeutung, den Prozess der Atmung, der, auch wenn alle anderen Vorgänge der Pflanze zum Stillstand kommen, noch bestehen bleibt, seinem eigentlichen Wesen nach zu verstehen, sondern man muss sich auch die Frage vorlegen, inwieweit er durch äussere Einflüsse in seinem Verlaufe modifiziert werden kann. Es sind nach dieser Richtung hin zahlreiche Untersuchungen angestellt und wertvolle Resultate gewonnen worden. Namentlich ist der Einfluss von Temperatur und Licht, Wassergehalt, CO_2- und O_2-Konzentration, Wasserstoffionenkonzentration und von der Verletzung der Pflanzen gründlich studiert und mit relativer Sicherheit festgestellt worden. Weniger zahlreich dagegen sind Untersuchungen über Veränderung der Atmung durch stoffliche Einflüsse, insbesondere durch Salze. Es ist daher physiologisch von Interesse, diesen Punkt durch genaue Methodik weiter aufzuklären.

Bevor wir jedoch zur Mitteilung unserer Versuchsresultate übergehen, mögen die über die oben genannte Frage bereits bekannten Tatsachen hier Erwähnung finden.

II. HISTORISCHES.

Schon 1869 hat A. MAYER nachgewiesen, dass gewisse Metallsalze, so z. B. CaCl₂, Eisen- Tonerde- und Mangansalz, die Entwicklung der

Hefe nicht wesentlich beeinflussen. KELLNER (1874) zeigte dann, dass
Erbsenkeimlinge, die in 0.5%iger KNO_3-Lösung kultiviert wurden,
energischer als im destill. Wasser atmen. Dann hat JACOBI (1899)
festgestellt, dass ausser KNO_3 auch Na- und Kaliumchlorid die Atmung
der submersen Pflanzen steigern, indem sie als Reiz wirken. Zahl-
reiche Untersuchungen über die Wirkungen der Elektrolyten auf die
Atmung der Pflanzen wurden in klarer Weise von PRIANISCHNIKOW
(1895), PALLADIN (1909), REINHARD (1910), ZALESKI (1910), KOSTYT-
SCHEW (1924), IWANOFF (1911), INMAN (1921), BROOKS (1920, 1921,
1922), LYON (1921), GUSTAFSON (1920-'21) und anderen gemacht.

Nach COOK (1926) geht bei Gegenwart schwerer Metallionen (Cu
und Ag) die Menge der Atmungskohlensäure bei *Aspergillus niger* ent-
weder sofort herab oder steigt zuerst an, um nachher zu fallen. Der
Eintritt der schädlichen Wirkung der Salze ist dabei konstant pro-
portional der Konzentration. Er nimmt dabei an, dass die Metalle
sich mit irgendeinem Bestandteile der Zellen verbinden und diese
Verbindung ihrerseits die Geschwindigkeitskonstanten der normalen
Atmungstätigkeit beeinflusst, wodurch die jeweilige Kohlendioxydabgabe
dementsprechend abgeändert wird. ANDRÉ MAYER und PLANTEFOL
(1926) haben die Wirkung der Elektrolyten und der Säuren auf die
Atmung von *Hypnum triquetrum* L. untersucht. Die Moose wurden
6 Stunden lang in Salzlösung mit der Konzentration $10^{-3}-1$ N bzw. 2
Stunden in Säuren mit der Konzentration bis 10^{-1} N getaucht und
nachher die Atmungsintensität durch Bestimmung des ausgeschiedenen
CO_2 ermittelt. Es ergab sich stets eine Änderung der Atmungsinten-
sität. Die Wirkung der Salze macht sich erst von einer bestimmten
Konzentration an geltend, und zwar wird die Atmung zuerst gesteigert,
sodass die Intensität den doppelten Wert der normalen erreichen kann.
Bei stärkerer Salzkonzentration nimmt die Intensität ab. Die Säuren
wirken in der gleichen Ordnung wie ihre Salze, nur wirken sie schon
in schwächerer Konzentration und innerhalb kürzerer Zeit. Im allge-
meinen ist die Wirkung immer die gleiche, doch bestehen für jede
Substanz Besonderheiten in ihrer Wirkungsweise.

Es wird nicht nur die Intensität, sondern auch die Natur der
Oxydation beeinflusst; der Atmungskoeffizient wird erst 1, wenn die
Atmungsintensität zum Maximum vergrössert wird. Übrigens dauert
die durch Elektrolyten auf die Atmung ausgeübte Wirkung ziemlich

lange, bisweilen mehrere Tage.

EMERSON ('27) zeigte dann, dass *Chlorella* wie andere heterotrophische Zellen atmet, wenn sie in Zuckerlösung unter Einwirkung von Blausäure, Schwefelwasserstoff und Kohlenmonooxyd gezüchtet wird.

Kürzlich studierte GENEVOIS ('27) die Atmung und Gärung bei grünen Pflanzen: *Chlorella pyrenoidea, Scenedesmus Basilliensis, Coelastrum proboscideum, Haematococcus pulvialis, Sticococcus basillaris* und *Pseudendoclonium Basilliense.* Dabei wurde die manometrische Methodik WARBURGs benutzt. Es ergab sich folgendes: Die Atmung von *Chlorella* wird unter Einwirkung natürlicher Zucker (Glukose, Fruktose, Mannose und Galaktose) bedeutend gesteigert; verhältnismässig stark wirken ferner Acetaldehyd, Natriumbutyrat, Alanin und andere Aminosäuren. Auffallend ist, dass Milchsäure und Brenztraubensäure nicht wirksam sind. Acetaldehyd steigert daneben den respiratorischen Quotienten erheblich; das beruht wahrscheinlich darauf, dass dabei Fettsäuren gebildet werden. Bei den höheren Aldehyden, Propylaldehyd und Valeraldehyd, war die Atmungssteigerung geringer. Verhältnismässig starke Steigerung der Atmung verursachen auch die Alkalisalze der niederen Fettsäuren, z. B. der Essigsäure, Buttersäure und Valeriansäure, wobei die Wirkung des Butyrats am grössten ist. Die verschiedenen Alkohole sind völlig unwirksam. Die anderen Organismen, sogar der grüne *Haematococcus* verhalten sich ähnlich, ausser *Pseudendoclonium* und der roten Dauerzelle von *Haematococcus.* Bei Abwesenheit von Sauerstoff erfolgt die Gärung in Zuckerlösungen: Die Kohlendioxydproduktion ist dabei etwa so gross wie bei der Atmung in mineralischer Lösung. Ausser bei dem blausäureempfindlichen *Scenedesmus* wird die Atmung der meisten Algen durch Blausäure in mineralischen Lösungen selbst in relativ hoher Konzentration nur wenig beeinträchtigt. In Gegenwart von Zucker oder der anderen obengenannten atmungsteigernden Stoffe wird hingegen die Atmung bei *Chlorella* stark gehemmt. Doch bleibt dabei auffallenderweise ein unempfindlicher Rest etwa von der Grösse der autotrophen Atmung übrig. Letztere bleibt also wahrscheinlich auch bei Zuckergegenwart als unvermindert nebenherverlaufender Vorgang erhalten. Durch Narkotika und oberflächenaktive Stoffe werden dagegen Atmung und Gärung gleichmässig gehemmt.

Meine vorliegenden Untersuchungen wurden auf Veranlassung und

unter Leitung von Herrn Prof. Dr. Y. YAMAGUTI im Verlaufe eines
akademischen Jahres, 1927-1928, im biologischen Institut der kaiser-
lichen Tôhoku Universität zu Sendai ausgeführt. Ich spreche auch an
dieser Stelle meinem hochverehrten Lehrer für seine vielfache Belehr-
ung und Anregung ⁻meinen herzlichen Dank aus. Es ist mir auch
eine angenehme Pflicht, Herrn Prof. Dr. S. HIBINO und dem ausser-
ordentlichen Prof. Herrn Dr. K. OKAZAKI† für ihre vielseitige Anreg-
ung meinen besten Dank auszudrücken.

III. Eigenschaften und Züchtung der Algen.

Als Versuchsobjekt wurde die Reinkultur einer einzelligen Alge,
Chlorella ellipsoidea benutzt. Die Reinkultur von *Chlorella* verdanke
ich Herrn NAKAJIMA. Die meisten Forscher begnügten sich mit einer
Rohkultur der Algen, deshalb möchte ich hier betonen, dass zu diesen
Versuchen eine absolute Reinkultur der Algen verwandt wurde. Als
Versuchsobjekt erwies sich diese Alge zweckmässig, weil sie schnell
wächst, unbeweglich ist und einen einfachen Entwicklungszyklus hat.
Die Alge wächst gut bei künstlicher Beleuchtung, sowohl auf Agar
als auch in reiner anorganischer Salzlösung. In organischer Nähr-
lösung gezüchtet, wird die Chlorophyllbildung schwächer und zum
Schluss gelbgrün.

Deshalb züchtete ich seit April in anorganischer Salzlösung unter
Beleuchtung einer Metallfadenlampe. Nach der Vorschrift von WAR-
BURG (1922) und seinem Mitarbeiter hielt ich mir folgende Stamm·
flüssigkeiten vorrätig :

I. $MgSO_4$ $7H_2O$ 50 g : 1000 ccm aus Glas gestill. Wassers. (0.2 molar)
II. KNO_3 25 g :   „   „ „   „   „   (0.25 molar)
III. KH_2PO_4 25 g :   „   „ „   „   „   (0.18 molar)
IV. $FeSO_4$ 2.8 g : „   „ „   „   „   (0.01 molar)

Das Gemisch von 100 ccm I, 100 ccm II, 100 ccm III und von 1 ccm
IV wurde mit Leitungswasser auf 1000 ccm aufgefüllt. Die Kultur-
flüssigkeit wird somit in bezug auf die in Frage kommenden Salze je
zu folgender Konzentration :

† Am 10. Januar dieses Jahres gestorben.

MgSO$_4$	0.02 molar.
KNO$_3$	0.025 molar.
KH$_2$PO$_4$	0.018 molar.
FeSO$_4$	0.00001 molar.

MOLISCH (1895), BENECKE (1907) und andere Forscher, die die Ansprüche der Algen an Mineralstoffen eingehend geprüft haben, konstatierten ein Ca-Bedürfnis nur bei *Spirogyra* und *Vaucheria*. Für die meisten Algen scheint Ca überflüssig zu sein. Deshalb entfernte ich die Ca-Salze aus der Nährlösung. Aller Wahrscheinlichkeit nach waren im Leitungswasser mehr oder weniger Ca-Salze vorhanden, obwohl mir die Analyse dafür fehlt.

Steriles Arbeiten ist nicht immer erforderlich, da in den anorganischen Nährflüssigkeiten, bei den hohen Beleuchtungsstärken und relativ grossen Aussaaten weder Bakterien noch andere Algen aufkommen.

Als Lichtquelle wurde eine Metallfadenlampe von 300 Watt Stromverbrauch in ein von fliessendem Kühlwasser

Fig. 1.

umgebenes Becherglas gehängt; ich liess die Lampe Tag und Nacht brennen. Um die Sedimentierung der Zelle zu verhindern, wurde ein langsamer Luftstrom dauernd durch die Kulturkolben, die ich für die Kultur von *Chlorella* konstruieren liess (Fig. 1), in einer Geschwindigkeit von ca. 60-70 Gasblasen pro Minute durchgeleitet. Die Einsaat pro Kolben betrug 0.75 ccm Zellen in ca. 700 ccm Kulturflüssigkeit. Die Lufttemperatur war im November, insbesondere in der Nacht, so niedrig geworden, dass die Algen vorwiegend Autosporen wurden. Dementsprechend wurde der Sauerstoffverbrauch der Algen sehr gering, sodass die Algen in einen Wasserthermostat von etwa 25 Grad gebracht werden mussten. Bei einer mässigen Temperatur von
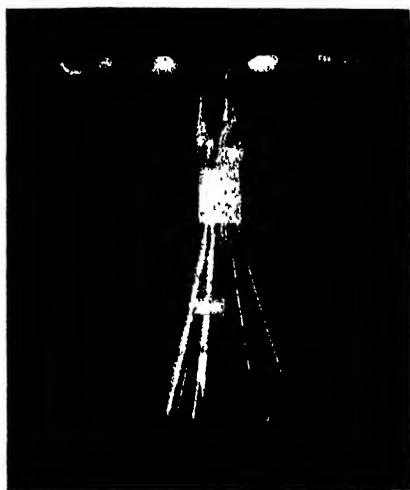
18°C. stieg die Zellenanzahl im Laufe einiger Tage auf das Mehrfache der Aussaat, wobei die anfangs hellgrüne Kulturflüssigkeit allmählich schwarzgrün wurde.

Nach Vermehrung auf das vier- bis fünffache wurden die Algen auf der Zentrifuge mehrmals mit frischer Nährlösung gewaschen, in einem graduierten Zentrifugierröhrchen gemesssen und dann in die Kolben eingesät. Wenn man *Chlorella* dauernd längere Zeit züchtet, insbesondere im Sommer, so verändert sich die Farbe, und die Alge, die sonst schwarzgrün ist, wird braun und setzt sich am Boden der Kolben ab; sodass die Nährlösung im Kulturkolben einmal alle zwei Wochen erneuert werden muss. Die zur Nachzucht bestimmten Zellen liess ich bei natürlichem Licht an einem Nordfenster stehen. In diesem Falle züchtete ich *Chlorella* in den von WINO-GRADSKY verwendeten Kolben (Fig. 2). Die zum Versuch bestimmten Zellen wurden im Gewächshaus eine Woche lang bestrahlt; sie vermehrten sich dabei auf etwa das vierfache. Trotz der Bewegung vermöge der Luftdurchleitung setzten sich die Algen allmählich am Boden der Kolben ab. Es genügt, sie im Laufe von 24 Stunden einmal aufzuwirbeln. Die Zellen verteilen sich bei leichtem Schütteln der Kolben sofort wieder.

Fig. 2.

IV. METHODIK.

I. APPARAT.

Zur Bestimmung des Sauerstoffverbrauchs gibt es zwei Methoden: die gasanalytische und manometrische. Ein Vorzug der manometrischen Methode ist es, dass wir bei ihr in kurzen Intervallen ablesen und so den „ Gang " der Prozesse beobachten können. Darum ist sie hier benutzt worden. Für diesen Zweck wurden vier Gefässe (Tröge) (Fig. 3) verwendet, die wir mit A, B, C und D bezeichnen. Ihr Rauminhalt einschliesslich Manometerkapillare bis zum Meniskus der

Sperrflüssigkeit beträgt ca. 20 ccm. Als Manometer kam der BARCROFT-sche Blutgasmanometer zur Verwendung, dessen Sperrflüssigkeit durch eine Schraube so reguliert wird, dass das Volumen während der Messung konstant bleibt. Die Manometerkapillare besitzt einen Durchmesser von 0.8 bis 1 mm. Als Sperrflüssigkeit ist BRODIEsche Lösung[1] benutzt worden, von der 10000 cmm 760 cmm von Quecksilber gleich kamen. Die Manometer sind an dem Wasserthermostaten montiert, dessen Temperatur hierbei 25°C beträgt und bis auf 0.05 Grad konstant gehalten werden konnte, und ihre Wände wurden mit schwarzem Packpapier der Trockenplatte beklebt.

Gefäss D dient als Thermobarometer. Es wird mit 2 ccm Salzlösung und 0.3 ccm KOH (5%) gefüllt. Gefäss A dient zur Bestimmung des Sauerstoffverbrauchs von *Chlorella*. Es wird mit 2 ccm Salzlösung, in dem sich 0.4 ccm *Chlorella* befindet, und 0.3 ccm KOH, das für die Absorption des ausgeschiedenen Kohlendioxyds dient, gefüllt. Gefäss B dient zur Bestimmung des Sauerstoffverbrauchs der Algen in dem doppeldestill. Wasser und Gefäss C zur Bestimmung des Sauerstoffverbrauchs von *Chlorella* in Nährlösung.

Fig. 3.

2 AUSFÜHRUNG DER MESSUNGEN

Die mehrere Tage lang in den Kulturkolben gezüchteten Algen wurden auf der Zentrifuge mehrmals mit doppeldestill. Wasser gewaschen, in einem gradierten Zentrifugierröhrchen gemessen und dann zweimal, je nach dem Zwecke, auf der Zentrifuge bei hoher Tourenzahl (2000 pro Minute) entweder mit Salzlösung, doppeldestill. Wasser

[1] Sie besteht aus 500 ccm Wasser, 23 g Natriumchlorid, 5 g gallensaurem Natron und ist zur Abhaltung von Bakterien mit so viel alkalischer Thymollösung versetzt, dass sie deutlich nach Thymol riecht.

oder mit Nährlösung gewaschen. Durch Schütteln des Algensediments in wässerigen Flüssigkeiten (namentlich in doppeldestill. Wasser, Salz- oder Nährlösungen) werden Suspensionen gewonnen, die sich mit der Pipette abmessen lassen.

Die Alge ist widerstandsfähig gegen mechanische und chemische Einwirkungen. Sie kann ohne Schädigung auf der Zentrifuge aus ihrer Nährlösung abgeschleudert und in andere Lösungen übertragen werden. Zum Versuche werden die Versuchsgefässe mit je 2 ccm Zellsuspension beschickt und durch Glasschliff mit dem Manometer verbunden; die Zellsuspension wird mit O_2 (worin 25 Vol-Proz. Stick- stoff) gesättigt, um die Luft aus dem Gasraum zu verdrängen. Mano- meter und Gefässe werden darauf in einen Wasserthermostat gehängt und die Hähne geöffnet, durch Bewegung der Exzenterscheibe mit einer Schwingungszahl von etwa 120 pro Minute 10 Minuten lang geschüttelt, bis Temperatur- und Druckgleichgewicht eingetreten ist. Vermehrung der Schüttelgeschwindigkeit bedingt keine Änderung der Ausschläge. Dann unterbricht man das Schütteln, stellt die Sperr- flüssigkeit des Manometers auf eine bestimmte Marke ein, schliesst die Hähne und löscht die Lampe aus. Alle Versuche wurden in der Dunkelheit ausgeführt.

Unter fortgesetztem Schütteln beobachtet man nunmehr die Druck- veränderungen; nachdem eine bestimmte Zeit verlaufen ist, unterbricht man das Schütteln, stellt die Sperrflüssigkeit auf ihren Anfangsstand ein und erhält so die Druckveränderungen bei konstantem Volumen, die durch den Ausschlag des Thermobarometers auf die Anfangstem- peratur und -aussendrücke korrigiert werden. Ist dies geschehen, so schüttelt man die Manometer wieder. Die korrigierten Druckverän- derungen mit den Gefässkonstanten (1.22- 1.28) multipliziert, geben den Sauerstoffverbrauch der Algen an.

V. Versuche mit Einzelnen Salzlösungen.

Als Elektrolyten sind die Chloride der Alkali- und der Erdalkali- metalle benutzt worden, um die Wirkung der Kationen zu vergleichen. Die sowohl als Nähr- wie auch als Reizstoffe dienenden Chemikalien stammten grösstenteils von MERCKS „garantiert reinen" Reagenzien. Zum Auslösen der Reizmittel benutzte ich doppeldestill. Wasser. Das

auf die übliche Weise gewonnene destill. Wasser wurde unter Zusatz von 1%igem Kalium Permanganat destilliert und dann von Glas zu Glas nochmals destilliert. Die Versuche wurden, mit Ausnahme nur einiger Fälle, wenigstens drei oder viermal wiederholt.

1. DIE WIRKUNG DER ALKALIKATIONEN.

a) Natriumionen.

Die Ergebnisse sind in folgenden Tabellen zusammengestellt.

TABELLE 1.

Laboratorium-Temperatur: 22.6°C.
Temperatur d. Wasserthermostats. 25°C.
Schwingungszahl 116 pro Minute.
Trog 1: 0.4 ccm *Chlorella* in 2 ccm 1 m. NaCl.
Trog 2: „   „   in 2 ccm H_2O.
Trog 3: „   „   in 2 ccm Nährlösung.

Zeit.	Verbrauchte Sauerstoffmenge in kmm.		
	1	2	3
Nach 10 Minuten.	7.63	17.28	17.63
15 „	11.25	26.30	25.00
30 „	27.37	51.72	48.62
45 „	43.62	88.47	73.62
60 „	59.87	105.77	96.12
75 „	72.24	129.89	114.74
90 „	87.11	154.04	135.91
105 „	103.36	181.61	157.11

TABELLE 2.

Laboratorium-Temperatur: 23°C.
Temperatur d. Wasserthermostats: 25°C.
Schwingungszahl 116 pro Minute.
Trog 1: 0.4 ccm *Chlorella* in 2 ccm 10^{-1}m NaCl.
Trog 2: „   „   in 2 ccm H_2O.
Trog 3: „   „   in 2 ccm Nährlösung.

Zeit.	Verbrauchte Sauerstoffmenge in kmm.		
	1		3
Nach 15 Minuten.	26.32	25.87	20.00
30 „	48.23	54.84	41.50
45 „	67.34	77.26	61.60
60 „	88.02	104.30	82.88
75 „	107.75	129.37	100.80
90 „	126.25	152.14	119.84

TABELLE 3.

Laboratorium-Temperatur: 22.5°C.

Temperatur d. Wasserthermostats: 25°C.

Schwingungszahl 116 pro Minute.

Trog 1: 0.4 ccm *Chlorella* in 2 ccm 10^{-2} m NaCl.

Trog 2:   „   „   in 2 ccm H_2O.

Trog 3.   „   „   in 2 ccm Nahrlösung.

Zeit.	Verbrauchte Sauerstoffmenge in kmm.		
	1	2	3
Nach 15 Minuten.	19.20	26.34	21.25
30 „	42.10	51.75	41.25
45 „	66.25	79.35	63.75
60 „	92.50	108.10	87.50
75 „	113.75	133.40	107. 0
90 „	137.53	159.85	128.75

TABELLE 4.

Laboratorium-Temperatur: 23°C.

Temperatur d Wasserthermostats: 25 C.

Schwingungszahl 116 pro Minute.

Trog 1· 0.4 ccm *Chlorella* in 2 ccm 10 ·m NaCl.

Trog 2:   „   „   2 ccm H_2O.

Trog 3:   „   „   in 2 ccm Nahrlösung.

Zeit.	Verbrauchte Sauerstoffmenge in kmm.		
	1	2	3
Nach 15 Minuten.	26.20	39.23	22.34
25 „	43.21	54.20	36.32
30 „	52.14	62.14	44.33
45 „	78.23	87.42	68.32
60 „	105.12	118.45	93.21
75 „	131.23	146.05	118.20
9) „	157.52	174.83	143.25

TABELLE 5.

Laboratorium-Temperatur: 23.7°C
Temperatur d. Wasserthermostats: 25°C.
Schwingungszahl 116 pro Minute.

Trog 1: 0.4 ccm *Chlorella* in 2 ccm 10^{-4} m NaCl.
Trog 2: „ „ in 2 ccm H_2O.
Trog 3: „ „ in 2 ccm Nährlösung.

Zeit.	Verbrauchte Sauerstoffmenge in kmm.		
	1	2	3
Nach 10 Minuten.	20.26	20.76	16.51
30 „	61.54	65.61	51.51
45 „	92.50	98.90	78.75
60 „	117.24	125.29	100.99
75 „	140.99	151.74	122.24
90 „	159.74	172.44	140.99

Aus den obigen Zahlen ersieht man, dass der Sauerstoffverbrauch der Algen sehr regelmässig vor sich geht, und dass er im doppeldestill. Wasser immer grösser als in der Nährlösung ist. Betr. der Ursache dieser Erscheinung vermutete Wo. OSTWALD (1910), dass destill. Wasser nur deshalb giftig sei, weil es dem Protoplasma Salze entziehe.

Ganz kürzlich hat es sich aber gezeigt, dass destilliertes Wasser kein Gift ist, vorausgesetzt, dass es von giftigen Beimischungen frei ist. In meinem Fall, wo dessen schädliche Wirkung auch nach der Befreiung von Beimischungen deutlich ist, muss diese Wirkung den giftigen Substanzen zugeschrieben werden, welche von *Chlorella* selbst infolge anomaler Lebenserscheinungen ausgeschieden werden und anfänglich äusserst erregend wirken. In 1 m. NaCl-Lösung ist aber die verbrauchte Sauerstoffmenge tatsächlich bedeutend geringer als in der Norm. In einer 10⁻¹ m-Lösung ist sie aber etwas grösser als in der Norm. In der 10^{-2} m- sowie 10^{-3} m-Lösung steigt der Sauerstoffverbrauch allmählich, und in der 10^{-1} m-Lösung wird er so gesteigert, dass er dem im doppeldestill. Wasser beinahe gleich wird. Diese Erscheinungen gelten wenigstens für alle von mir geprüften Elektrolyten und für die Versuchsdauer von 3 Stunden.

Um diese Verhältnisse anschaulich zu machen, nahm ich den Sauerstoffverbrauch von *Chlorella* in Nährlösung als 100 Proz. (Norm) und gab denjenigen bei verschiedenen Mollösungen von NaCl im Prozentsatz zur Norm an.

Die dabei erhaltenen Resultate sind in Fig. 4 wiedergegeben. Aus dieser Figur ist ersichtlich, dass bei den meisten Mollösungen der Sauerstoffverbrauch der Algen nach Ablauf von 90 Minuten nicht so deutliche Unterschiede zeigt. Aber man erkennt die Neigung des Sauerstoffverbrauchs der Algen, in den 10^{-2} m-, 10^{-3} m- sowie 10^{-4} m-
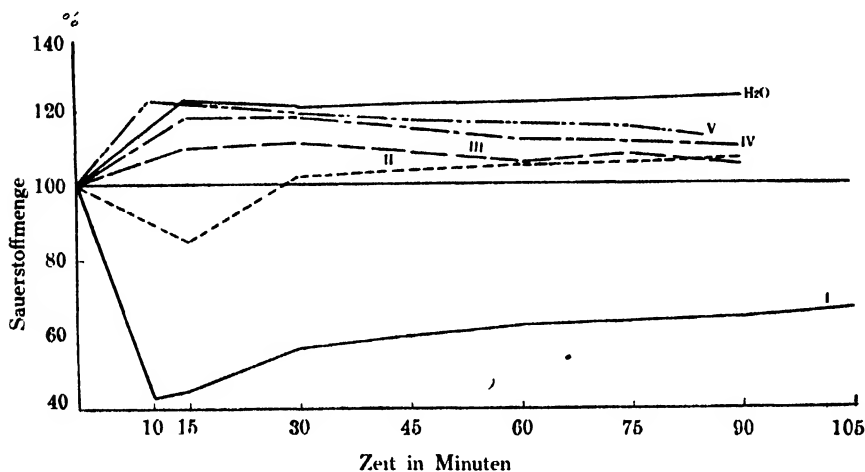
Fig. 4. *NaCl·* I 1 M, II 10^{-1} M, III 10^{-2} M, IV 10^{-3} M, V 10^{-4} M.

Lösungen in den ersten 10–15 Minuten plötzlich zu steigen, um dann allmählich auf eine bestimmte Prozentzahl zu fallen, während bei der 1 m- und 10^{-1} m- Lösung die Kurven des Sauerstoffverbrauchs den anderen entgegengesetzt verlaufen.

b) Kaliumionen.

Was die Wirkung der Kaliumionen anbetrifft, so ergibt sich, dass in der 1 m-Lösung die verbrauchte Sauerstoffmenge geringer als die Norm ist, wie das beim Na-Ion der Fall ist; in der 10^{-1} m-Lösung ist sie geringer als die in der Nährlösung, im Gegensatz zum Versuche mit NaCl, wo sie in der genannten Konzentration grösser als die Norm war. Erst in der 10^{-2}-Lösung steigt sie über diejenige bei der Nährlösung.

In der 10^{-4} m-Lösung wird der Sauerstoffverbrauch beinahe gleich demjenigen beim doppeldestill. Wasser.
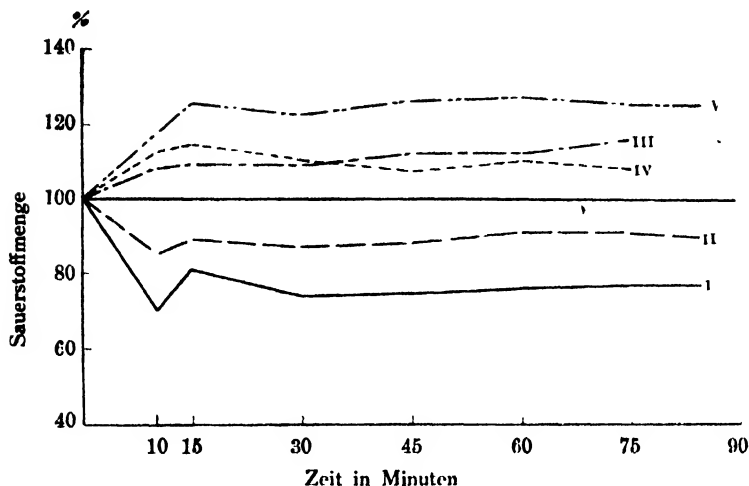
Fig. 5. *KCl·* I 1 M, II 10⁻¹ M, III 10⁻² M, IV 10⁻³ M, V 10⁻⁴ M.

Um diese Verhältnisse des Sauerstoffverbrauchs von *Chlorella* in den verschiedenen Mollösungen von KCl anschaulich zu machen, sind die Resultate im Prozentsatz zu dem bei der Nährlösung in Fig. 5 graphisch zusammengestellt.

c) Rubidiumionen.

Die Wirkungen des Rb-Ions sind stärker als die der Natrium- und Kaliumionen. Sowohl bei den Fällen von 10^{-1} m-, 10^{-2} m- als auch
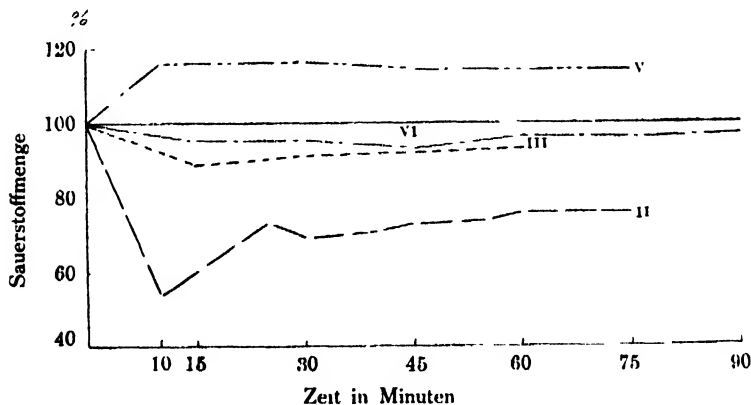
Fig. 6. *RbCl*: II 10⁻¹ M, III 10⁻² M, IV 10⁻³ M, V 10⁻⁴ M.

von 10^{-3} m- Lösungen ist der Sauerstoffverbrauch dieser Algen viel geringer als der in der Nährlösung. Und erst in der 10^{-4} m-Lösung steigt er über den bei der Nährlösung. Die Resultate, umgerechnet auf den Prozentsatz derjenigen bei der Nährlösung, sind in Fig. 6 wiedergegeben.

d) Lithiumionen.

Die Versuche über die Wirkung des Lithiumions auf den Sauerstoffverbrauch von *Chlorella* ergeben, dass das Lithiumion im allgemeinen auf den Sauerstoffverbrauch dieser Algen schädlich, jedoch nicht so schädlich wie Rb-Ion wirkt. In der 10^{-1} m-Lösung ist der Sauerstoffverbrauch nach Ablauf von 90 Minuten etwas grösser als derjenige in der Nährlösung, während er sich in der 10^{-4} m-Lösung vergrössert und beinahe gleich demjenigen im doppeldestill. Wasser wird.

Um diese Verhältnisse anschaulich zu machen, sind die Ergebnisse, prozentual zu denjenigen bei der Nährlösung umgerechnet, graphisch dargestellt worden. (Fig. 7). Aus dieser Figur ersieht man, dass in
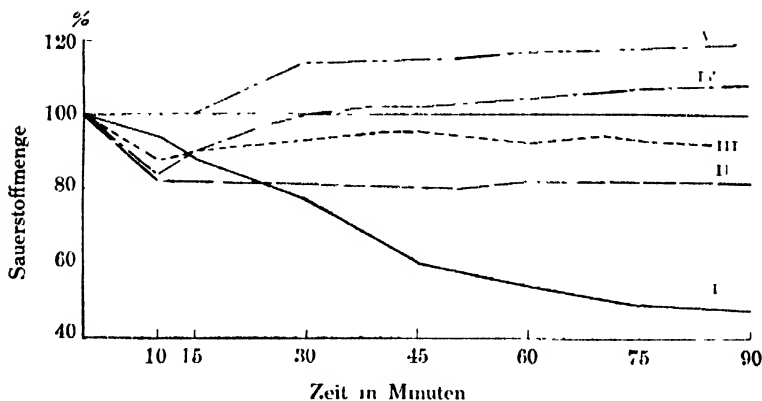
Fig. 7. *LiCl* I 1 M, II 10⁻¹ M, III 10^{-2} M, IV 10⁻³ M, V 10⁻⁴ M.

der 1 m-Lösung der Sauerstoffverbrauch ca. 50 Proz. der Norm beträgt. Und in der 10^{-1} m-Lösung steigt er bis zu 80 Proz., in der 10^{-2} m-Lösung bis zu 92 Proz. und erst in der 10^{-3} m- und 10^{-4} m-Lösung bis auf 108 bezw. 120 Proz. der Norm.

Um die Wirkung der verschiedenen Alkalikationen, namentlich von Na, K, Li und Rb, miteinander vergleichen zu können, sind die Resultate der Versuche mit verschiedenen Ionen nach Ablauf von 90
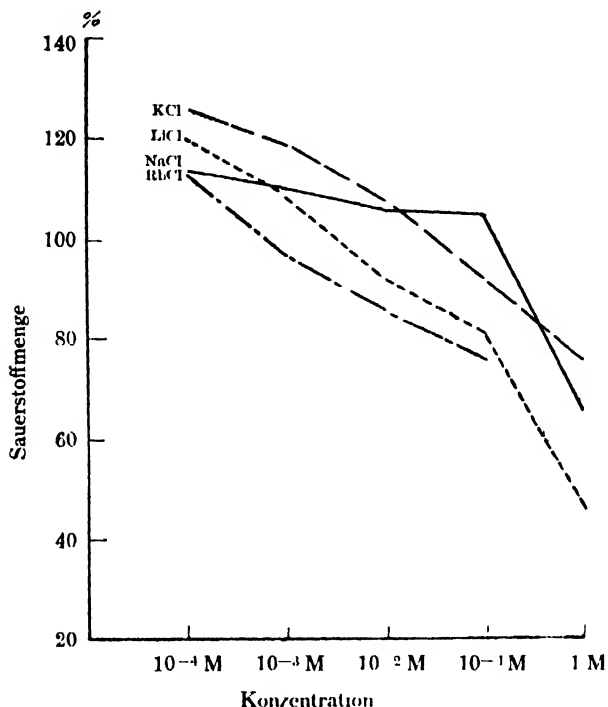
Fig. 8. Wirkung verschiedener Alkalikationen auf den O_2-Verbrauch

Minuten in Fig. 8 graphisch zusammengestellt. Aus dieser Figur sind zwischen allen einzelnen Alkalikationen in bezug auf die schädliche Wirkung auf den Sauerstoffverbrauch folgende Reihen zu entnehmen.

Bei der 1 m-Lösung. K Na<Li

„ „ 10^{-1} m-Lösung. Na<K<Li<Rb

„ „ 10^{-2} m-Lösung. K<Na<Li<Rb

„ „ 10^{-3} m-Lösung. K Na<Li<Rb

„ „ 10^{-4} m-Lösung. K<Li<Na<Rb

Aus diesen Ergebnissen kann man schliessen, dass im grossen und ganzen die Reihenfolge der schädlichen Kationenwirkungen auf den Sauerstoffverbrauch von *Chlorella*, wie folgt, ist: K<Na<Li Rb.

2. DIE WIRKUNG DER ERDALKALIKATIONEN.

Zunächst muss man die Wirkung der Erdalkalikationen, namentlich von Ba, Sr, Mg und Ca, auf den Sauerstoffverbrauch dieser Algen erkennen.

a) Bariumionen.

Die Versuchsergebnisse sind in der folgenden Figur in üblicher Weise graphisch dargestellt worden (Fig. 9). In der 1 m-Lösung sinkt
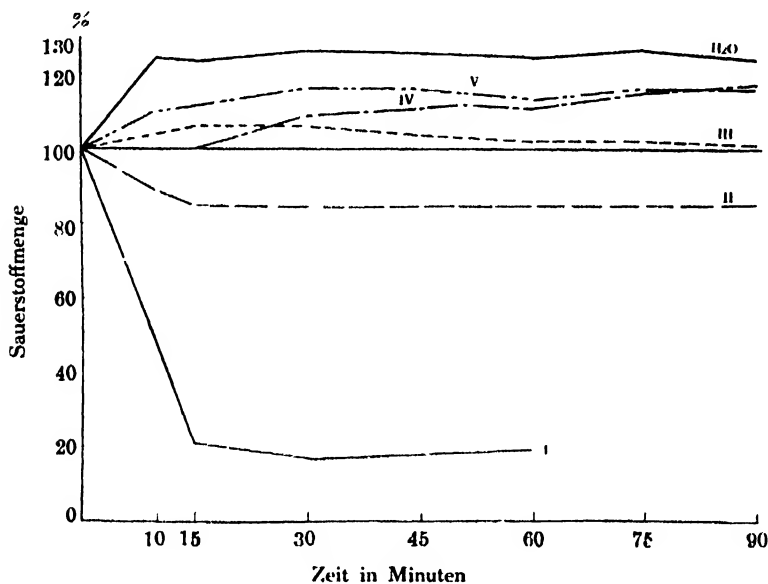
Fig. 9. $BaCl_2$: I 1 M, II 10^{-1} M, III 10^{-2} M, IV 10^{-3} M, V 10^{-4} M.

die Menge des aufgenommenen Sauerstoffs auffallend, aber in der 10^{-1} m-Lösung beträgt sie 85 Proz. der Norm. In der 10^{-2} m-Lösung steigt sie im Laufe von 15 Minuten plötzlich und sinkt dann allmählich bis zur Norm.

In der 10^{-3} m-Lösung ist sie während der ersten 10 Minuten der Versuche etwas gering, steigt aber allmählich ganz wie bei der 10^{-4} m-Lösung, indem sie bei den Lösungen zum Schluss ein gleiches Niveau erreicht.

b) Strontiumionen.

In bezug auf die Wirkung der Strontiumionen auf den Sauerstoff-verbrauch von *Chlorella* habe ich gefunden, dass in der 10^{-1} m-Lösung die verbrauchte Sauerstoffmenge jedenfalls geringer als die Norm ist, indem sie anfangs bedeutend kleiner, jedoch nach Ablauf von 40 Minuten etwas grösser als diejenige bei der entsprechenden Lösung von Ba wird.

In der 10^{-2} m-Lösung ist die verbrauchte Sauerstoffmenge grösser als diejenige bei der entsprechenden Lösung von Bariumn und der Nährlösung.

Weiter bemerkt man, dass sie in den 10^{-1} m- und 10^{-1} m-Lösungen
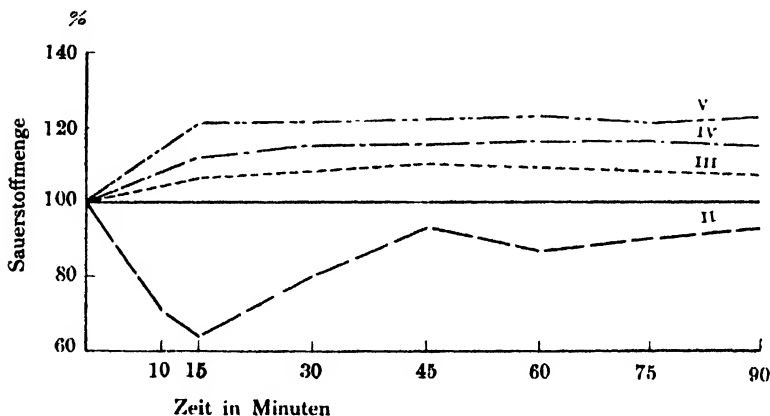
Fig. 10. *SrCl₂*: II 10^{-1} M, III 10^{-2} M, IV 10^{-3} M, V 10^{-4} M

sehr gross und beinahe gleich derjenigen beim doppeldestill. Wasser ist. Die beistehende Figur gibt das Verhältnis der obigen Resultate wieder.

c) Magnesiumionen.

Was nun die Wirkung des Magnesiumions auf den Sauerstoffver-brauch von *Chlorella* anbetrifft, so sind die Ergebnisse in üblicher Weise in Fig. 11 wiedergegeben.

Wie man aus nebenstehender Figur ersieht, ist der Sauerstoffver-brauch in der 1 m-Lösung erheblich geringer im Vergleich zu dem in
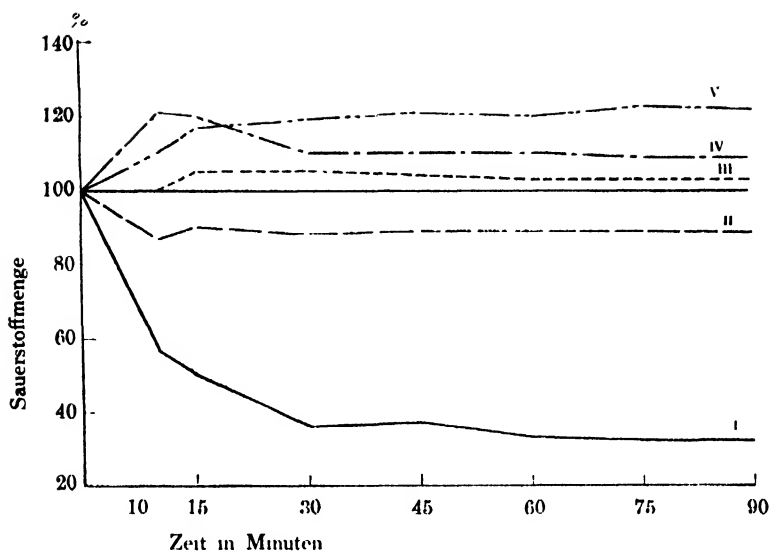
Fig. 11. $MgCl_2$. I 1 M, II 10^{-1} M, III 10 $^{-2}$ M, IV 10^{-3} M, V 10 $^{-1}$ M.

der Nährlösung und zwar beträgt er nur 32 Proz. der Norm.

In der 10^{-1} m-Lösung ist er geringer als die Norm, doch grösser als derjenige bei der entsprechenden Lösung von Barium, sodass das Magnesium in dieser Hinsicht zwischen Strontium und Barium steht. Erst in der 10^{-2} m-Lösung wird die verbrauchte Sauerstoffmenge durch die Algen 1.03 mal, in der 10^{-3} m-Lösung 1.1 mal und in der 10^{-1} m-Lösung 1.22 mal so gross wie diejenige in der Nährlösung.

d) Calciumionen.

Was endlich die Wirkung des Ca-Ions anbetrifft, so ergeben die Versuche, dass der Sauerstoffverbrauch der Algen nicht nur in den 10^{-1} m-, 10^{-2} m, 10 $^{-1}$ m-Lösungen, sondern auch in der 10 $^{-4}$ m-Lösung ausnahmlos geringer als derjenige in der Nährlösung ist.

Um diese Verhältnisse klar zu machen, sind die Resultate in üblicher Weise in Fig. 12 zusammengestellt worden.

In der 10^{-1} m-Lösung nimmt der Sauerstoffverbrauch der Algen nach anfänglich schroffem Sinken allmählich ab, und zwar beträgt er nach Ablauf von 90 Minuten nur noch 23 Proz. der Norm. In den
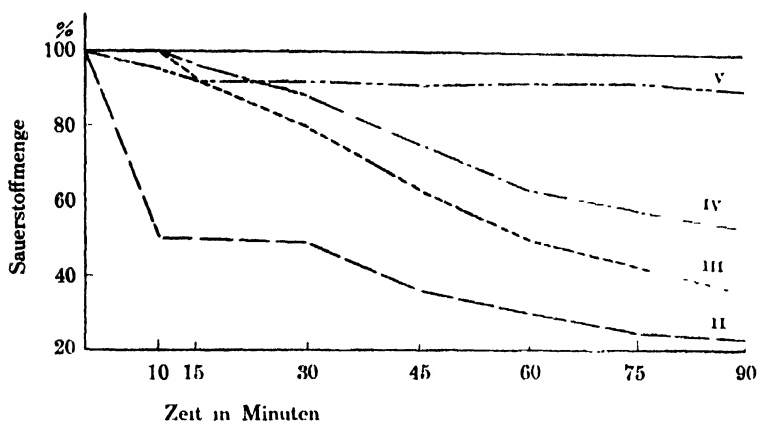
Fig. 12. $CaCl_2$ · II 10^{-1} M, III 10^{-2} M, IV 10^{-3} M, V 10^{-4} M.

10^{-2} m- und 10^{-3} m-Lösungen nimmt die Menge des verbrauchten Sauerstoffs in den erften 10 Minuten der Versuche etwas langsam, danach steiler ab, sodass sie nach Ablauf von 90 Minuten nur 36 bzw. 53 Proz. der Norm beträgt. Selbst in der 10^{-4} m-Lösung beträgt sie nur 90 Proz. derjenigen bei der Nährlösung.

Um nun die Wirkungen verschiedener Erdalkalikationen, namentlich von Ba, Sr, Mg und Ca, auf den Sauerstoffverbrauch dieser Algen zu vergleichen, sind die obigen Resultate in Fig. 13 zusammengestellt, die in allen Verhältnissen der Fig. 8 entspricht.

Auffallend und merkwürdig ist es, dass die Kurve der Calciumionen im Gegensatz zu den anderen ganz verschieden und einseitig ausgefallen ist. In bezug auf die schädliche Wirkung der verschiedenen Ionen ergeben sich die folgenden Reihen:

Bei der 1 m-Lösung. Mg<Ba.

„ „ 10^{-1} m-Lösung. Sr ⊂Mg<Ba<Ca

„ „ 10^{-2} m-Lösung. Sr ⊂Mg<Ba<Ca

„ „ 10^{-3} m-Lösung. Ba<Sr<Mg<Ca

„ „ 10^{-4} m-Lösung. Sr⊂Mg<Ba ⊂Ca

Aus diesen Resultaten kann man schliessen, dass die Reihenfolge der schädlichen Kationenwirkung auf den Sauerstoffverbrauch von *Chlorella* im grossen und ganzen so anzugeben ist: Sr<Mg<Ba<Ca.
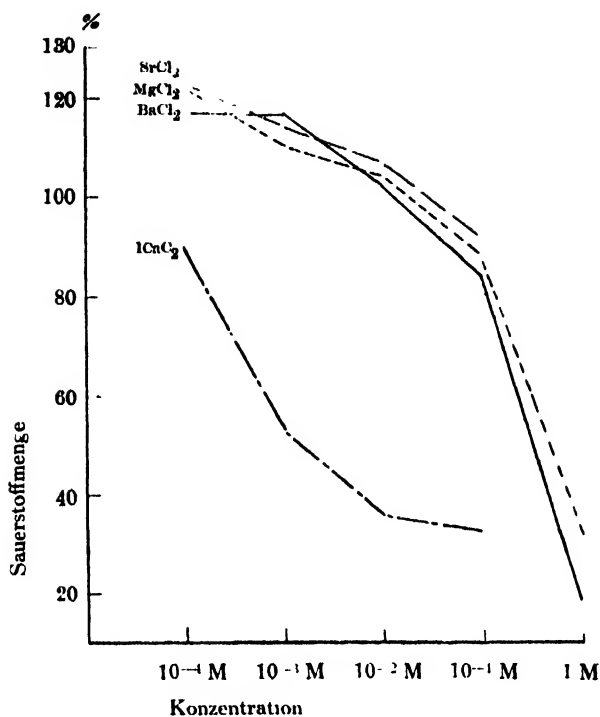
Fig. 13. Wirkung verschiedener Erdalkalikationen auf die O_2-Aufnahme.

Aus diesen Versuchen mit den verschiedenen Alkali- und Erdalkali-kationen geht also hervor, dass alle hier benutzten Kationen in reiner Lösung auf den Sauerstoffverbrauch von *Chlorella* schädlich wirken, so dass sie in sehr verdünnter Lösung die Atmung beschleunigen können. Die scheinbare Ausnahme des Calciums beruht höchstwahr-scheinlich darauf, dass das Calcium am schädlichsten auf die Atmung dieser Pflanze wirkt, sodass die hier genommene Verdünnung vielleicht noch zu klein war, um die beschleunigende Wirkung nachzuweisen.

VI. ANTAGONISTISCHE IONENWIRKUNG AUF DIE O_2-AUFNAHME.

Über die antagonistischen Ionenwirkungen auf die biologischen Systeme wurden schon zahlreiche Untersuchungen ausgeführt. Um die genannte antagonistische Kationenwirkung in bezug auf den Sauer-

stoffverbrauch bei *Chlorella* zu erkennen sind die folgenden Versuche ausgeführt worden. Die in den folgenden Zeilen mitgeteilten Ergebnisse sind aber meistenteils noch unvollständig, u. a. ist der Punkt, wo der vollkommene Antagonismus zwischen den betreffenden Ionen erzielt werden könnte, infolge verschiedener Mischverhältnisse noch nicht festgestellt worden, sodass die folgenden Mitteilungen noch nichts Endgültiges bringen.

<div align="center">

I   ANTAGONISMUS ZWISCHEN EIN- UND
ZWEIWERTIGEN IONEN.

a.   *Antagonismus zwischen Ca- und Alkalikationen.*

</div>

Die Ergebnisse in bezug auf den Antagonismus zwischen Ca- und einwertigen Kationen sind kurz in Fig. 14 zusammengestellt worden.

Hier sei bemerkt, dass in dieser und in den folgenden Figuren die Ergebnisse immer in den Prozentzahlen der Nährlösung angegeben worden sind.
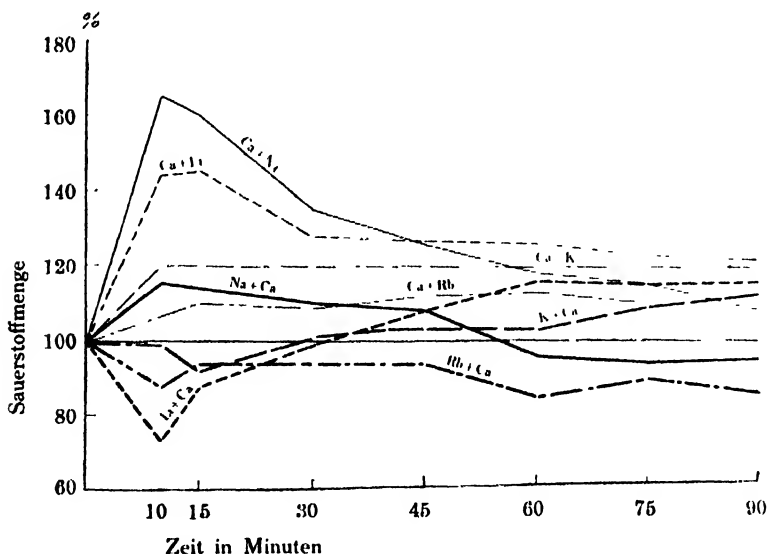
Fig. 14.   Wirkung gemischter Lösungen von Ca- u. Alkalisalzen.

Aus **Fig. 14** ist ersichtlich, dass im Gemisch von Na und Ca (90 ccm 10^{-3} m NaCl + 10 ccm 10^{-1} m CaCl$_2$) die verbrauchte O$_2$-Menge

anfangs grösser, nach Ablauf von 60 Minuten kleiner als diejenige der Kontrolle ist, indem sie schliesslich ca. 86% beträgt, im Gegensatz zur reinen 10^{-3}NaCl-Lösung, wo sie 110% der Kontrolle beträgt. Im umgekehrten Mischverhältnis (90 ccm $CaCl_2 + 10$ ccm NaCl) verhält sich das System merkwürdig, indem die Gegenwart von Na in kleiner Menge die O_2-Aufnahme stark beschleunigt und in den ersten 10 Minuten ein Maximum (165%) erreicht, um danach gleichmässig zum Werte der reinen NaCl-Lösung herabzusinken.

Was die antagonistische Wirkung zwischen Ca und Na in bezug auf die Atmungsprozesse anbelangt, so finden sich nicht wenige Angaben darüber in der Literatur. Z. B. ist sie bei *Bac. subtilis* (BROOKS, 1920), bei *Aspergillus niger* (GUSTAFSON, 1920), bei *Erodea canadensis* (LYON, 1921) und bei anderen Pflanzen nachgewiesen worden. Ferner sind ähnliche Verhältnisse in bezug auf das Wachstum von Weizen (REED, 1918, OSTERHOUT, 1905), die Permeabilität der Plasmahaut (OSTER-HOUT, 1905), die Strömung und andere Eigentümlichkeiten des Plasmas (CHOLODONYJ, 1923), und auf den Geotropismus (CHOLODONYJ, 1923) festgestellt worden.

Zunächst bleibt beim System K + Ca im Gemisch von 90 ccm 10^{-3} m KCl + 10 ccm 10^{-3} m $CaCl_2$ die Menge des verbrauchten Sauerstoffs während der ersten 30 Minuten unter die Norm herabgesetzt, doch danach neigt sie dazu, Schritt für Schritt bis zum Werte bei reiner 10^{-3} KCl Lösung zu steigen. Aus dieser Tatsache ist leicht zu entnehmen, dass beim K etwas mehr Ca als beim Na zugesetzt werden muss, um die betreffende schädliche Wirkung zu neutralisieren. Im umgekehrten Mischverhältnis wirkt dagegen die Gegenwart einer kleinen Menge K auf die O_2-Aufnahme beschleunigend, indem die Kurve in den ersten 10 Minuten die Höhe von 120% der Kontrolle erreicht und danach der Grundlinie parallel verläuft.

Betreffs dieses Antagonismus liegt eine ähnliche Tatsache beim Längenwachstum der Weizenwurzel (OSTERHOUT, 1905), bei der Änderung der Plasmaeigenschaften (CHOLODONYJ, 1923) und auch bei der Ammonifikation von *Bacillus subtilis* (LIPMAN, 1909) vor.

Beim Gemisch von Li und Ca (90 ccm 10^{-3} m LiCl + 10 ccm 10^{-3} m $CaCl_2$) ist, wie aus der Figur ersichtlich, die Prozentzahl der verbrauchten Sauerstoffmenge in den ersten 10 Minuten unter sämt-lichen hier benutzten Gemischen am kleinsten, trotzdem sie allmählich

wächst, um nach Ablauf von 90 Minuten grösser als die bei der reinen 10^{-3} m LiCl-Lösung zu werden. Drum ist es einleuchtend, dass die schädliche Wirkung des Li-Ions durch Zusatz von Ca-Ionen neutralisiert werden kann. Dagegen läuft die Kurve für das Gemisch von Ca(9) + Li(1) grade entgegengesetzt zu der des vorigen Falls, sodass das Li zuerst stark anregend wirkt, indem die O_2-Aufnahme nach Ablauf von 90 Minuten, im Gegensatz zum Fall des vorigen Mischverhältnisses, unter sämtlichen am grössten war. Deshalb ist die Annahme berechtigt, dass der Antagonismus zwischen Ca und Li am stärksten ist.

Ein schwacher Antagonismus zwischen Li und Ca ist auch von SAKAMURA (1924) in bezug auf die Beweglichkeit und den Zusammenhang der Kolonie von *Gonium* mitgeteilt. Nach einer neueren Untersuchung von REZNIKOFF (1927) über das Protoplasma von *Amoeba dubia* nimmt die antagonistische Wirkung von $CaCl_2$ beim Plasmalemma in der Reihenfolge von Li>Na>K weg, und beim inneren Protoplasma fällt sie in der Reihenfolge von K >Na>Li aus.

Endlich bleibt im System von Rb + Ca (90 ccm 10^{-3} m RbCl + 10 ccm 10^{-3} m $CaCl_2$) die Prozentzahl der verbrauchten O_2-Menge immer unter der Norm, jedenfalls mit kleinen Schwankungen, und schliesslich beträgt sie 86% der Norm. In der umgekehrten Kombination von Ca(9) + Rb(1) wird die O_2-Aufnahme durch Gegenwart einer kleinen Menge Rb beschleunigt, doch bleibt die Beschleunigung unter den sämtlichen Alkalikationen am schwächsten. Deshalb ist der Schluss berechtigt, dass zwischen Ca und Rb auch ein Antagonismus besteht.

Wenn man bloss aus diesen Ergsbnissen betr. der Stärke der antagonistischen Wirkung des Ca auf die oben genannten Alkalikationen eine Folgerung ziehen soll, so wäre nach dem Verlauf der Kurven etwa die folgende Reihenfolge als gültig anzusehen:

$$Li>K>Na>Rb$$

b. *Antagonismus zwischen Ba- und Alkalikationen.*

Die den vorigen ganz entsprechenden Versuche mit Ba und Alkalikationen ergaben die Resultate, die in üblicher Weise in Fig. 15 graphisch zusammengestellt worden sind. Aus Fig. 15 kann man entnehmen, dass das Ba als antagonistisches Reagenz in folgender Reihenfolge wirksam ist: K>Na>Rb>Li.
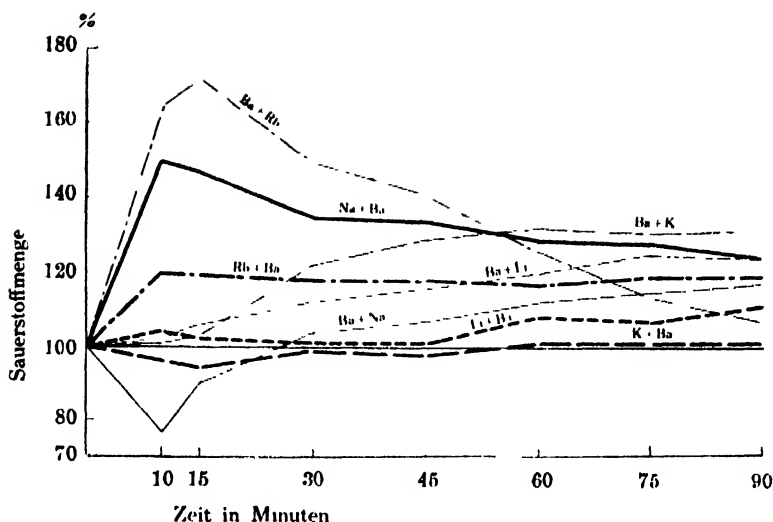
Fig. 15. Wirkung gemischter Lösungen von Ba- u. Alkalisalzen.

c. *Antagonismus zwischen Mg- und Alkalikationen.*

Was nun den Antagonismus zwischen Mg und einwertigen Kationen anbelangt, so kann man etwa aus der graphischen Darstellung in Fig.
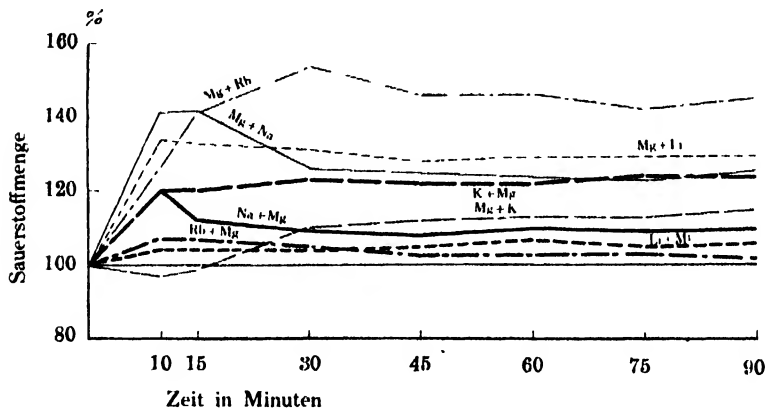
Fig. 16. Wirkung gemischter Lösungen von Mg- u Alkalisalzen.

16 entnehmen, dass das Mg in der folgenden Reihenfolge gegen Alkalikationen antagonistisch wirksam ist: K>Na>Li>Rb.

d. *Antagonismus zwischen Sr und Alkalikationen.*

Was endlich die antagonistische Wirkung von Sr auf die Alkalika-
tionen anbetrifft, so sind die Ergebnisse in Fig. 17 graphisch zusam-
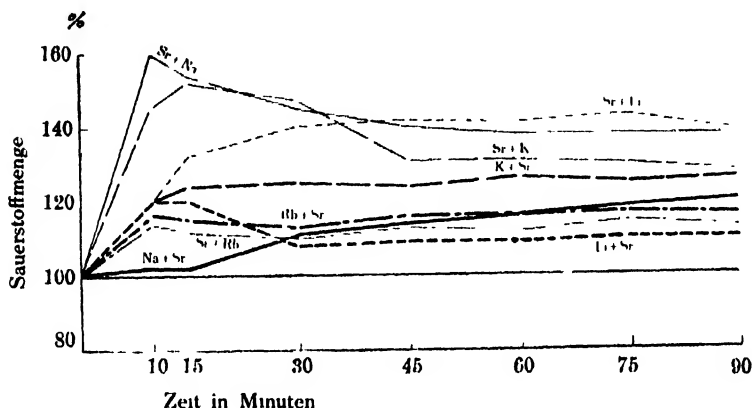
Fig. 17. Wirkung gemischter Lösungen von Sr u. Alkalisalzen.

mengestellt worden. Daraus kann man schliessen, dass das Sr in der
folgenden Reihenfolge antagonistisch wirksam ist: K>Na>Rb>Li.

2. ANTAGONISTISCHE WIRKUNG DER EINWERTIGEN KATIONEN UNTEREINANDER.

Die Versuchsergebnisse sind in den Figuren 18 und 19 in üblicher
Weise zusammengestellt worden.

Aus Fig. 18 ist ersichtlich, dass im Gemisch von K und Na (90 ccm
10^{-3} m KCl + 10 ccm 10^{-3} NaCl) die Prozentzahl der aufgenommenen
O_2-Menge jedenfalls grösser als die der Kontrolle ist, indem sie in
den ersten 10 Minuten ihr Maximum von 125% erreicht. Ganz all-
mählich sinkt sie ein wenig, sodass sie nach Ablauf von 90 Minuten
der der reinen Lösung beinahe gleich ausfällt. Beim umgekehrten
Mischverhältnis (90 ccm Na + 10 ccm K) wirkt das Gemisch auf die
O_2-Aufnahme ganz anders als das vorige. Während der ersten 15
Minuten nimmt die aufgenommene O_2-Menge enorm zu, und erreicht
ein Maximum von 165%. Danach nimmt sie allmählich ab, doch
beträgt sie beim Abschluss des Versuchs noch 151% der Norm.
Demnach ist klar, dass das K die beschleunigende Wirkung von Na
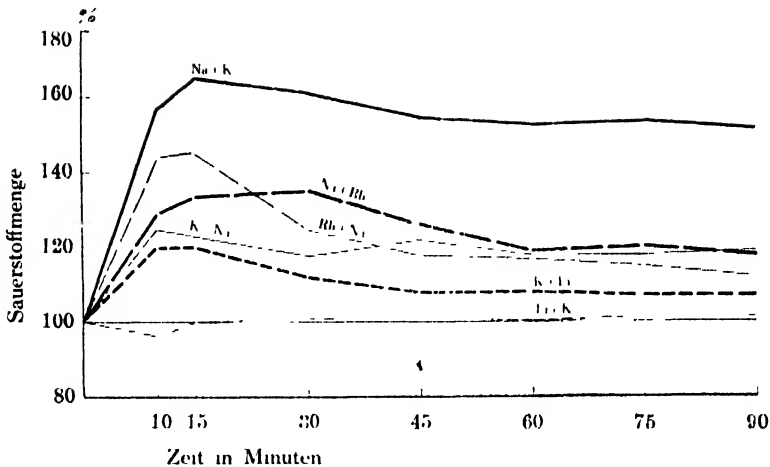
Fig. 18. Wirkung gemischter Salzlösungen von Na u. K, Na u. Rb, u K u. Li.

auf die Atmung weiter enorm verstärken kann, im Gegensatz zum Fall des umgekehrten Mischverhältnisses, wo die deutlich verstärkende Wirkung von Na auf K ausbleibt. Möglich, sogar sehr wahrscheinlich, daß diese Erscheinung auf der kombinierten Wirkung von K und Na, die LOEB (1911) „Salzeffekt" genannt hat, beruht.

Im Gemisch von Rb und Na (90 ccm 10^{-3} m RbCl + 10 ccm 10^{-3} m NaCl) steigt plötzlich die Prozentzahl der verbrauchten O_2-Menge in den ersten 10–16 Minuten, um nachher bis zum Werte bei reiner NaCl-Lösung zu sinken. Man erkennt also, dass die Wirkung des Rb-Ions auf die O_2-Aufnahme durch Zusatz von Na-Ion gesteigert wird. Im umgekehrten Mischverhältnis der genannten Ionen, d. h. 90 ccm Na + 10 ccm Rb, kam im Gegenteil fast gar keine augenscheinliche Änderung der aufgenommenen O_2-Menge zum Vorschein.

Zunächst fällt bei der Kombination von K und Li die absorbierte O_2-Menge im Gemisch von 90 ccm 10⁻ m KCl + 10 ccm 10⁻ m LiCl nach anfänglicher Steigerung zum Schluss kleiner als bei reiner KCl-Lösung aus. Im umgekehrten Mischverhältnis ist aber zwischen den beiden Ionen ein Antagonismus deutlich, indem die aufgenommene O_2-Menge während der ganzen Versuchsdauer der der Kontrolle beinahe gleich war.

Wie man aus Fig. 19. ersehen kann, steigt bei der Kombination von 90 ccm KCl + 10 ccm RbCl die Atmung in den ersten 30 Minuten
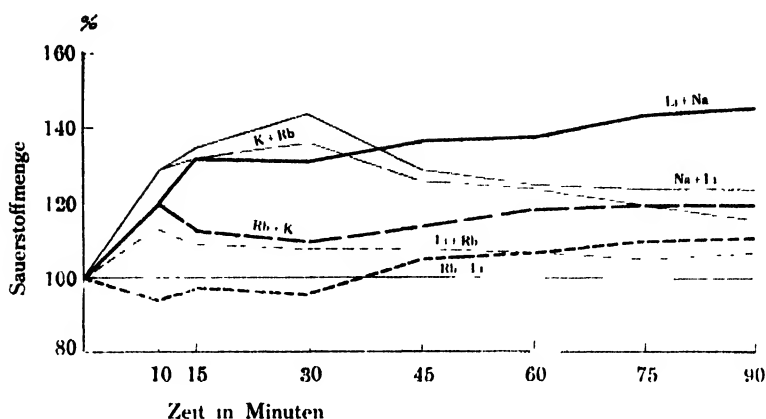
Fig. 19. Wirkung gemischter Salzlosungen von K u. Rb, Li u. Rb, u Na u. Li.

über die bei reiner KCl-Lösung, um dann bis zur Menge bei letzterer zu sinken. Im Gegensatz dazu wird beim System im umgekehrten Mischverhältnis die schädliche Wirkung von Rb auf den O_2-Verbrauch durch Gegenwart von K abgeschwächt, sodass die aufgenommene O_2-Menge nach Ablauf von 90 Minuten etwas kleiner als die bei der reinen KCl-Lösung ausfällt.

Zunächst ist im Gemisch von 90 ccm LiCl + 10 ccm NaCl die beschleunigende Wirkung von Na auffallend; beim Abschluss des Versuchs beträgt sie 145% der Kontrolle. Der Versuch mit dem umgekehrten Mischverhältnis ergab aber während der ersten 30 Minuten eine maximale Beschleunigung des O_2-Verbrauchs; beim Abschluss des Versuchs betrug die aufgenommene O_2-Menge 121% der Kontrolle, die jedenfalls grösser als die bei reiner NaCl-Lösung ist.

Was zuletzt die gegenseitige Wirkung von Li und Rb anbetrifft, so ist aus Fig. 19 zu entnehmen, dass im Gemisch von 90 ccm RbCl + 10 ccm LiCl die absorbierte O_2-Menge während der ganzen Versuchsdauer entsprechend der der reinen Rb-Lösung wächst, sodass die beschleunigende Wirkung der Li-Ionen auf die O_2-Aufnahme der Algen auffällig ist. Im Gegensatz dazu tritt beim umgekehrten Mischverhältnis eine ähnliche Wirkung von Rb nur im Anfang des Versuchs hervor und bleibt durchweg konstant auf der Höhe der reinen Li-Lösung.

Diese Ergebnisse werden besser verständlich, wenn man hier auf

die im vorigen Abschnitt angegebene Kationenreihe : K<Na<Li<Rb
Rücksicht nimmt, weil der Stand je zweier Glieder in dieser Reihe
ausschlaggebend dafür zu sein scheint, wie sich das einzelne Kation
im Gemisch mit irgendeinem anderen verhalten soll. Mit anderen
Worten, beim Zusatz einer kleinen Menge wirkt das Kation links in
der Reihe anregend und das rechts in der Reihe hemmend für die
anderen ; jedoch wird dabei nicht deutlich, ob die beiden Ionen, je
mehr sie sich von einander entfernen, umso wirksamer aufeinander
sind.

3   ANTAGONISTISCHE WIRKUNG DER ZWEIWERTIGEN IONEN UNTEREINANDER

Die Versuchsergebnisse mit den Kombinationen von Mg +Sr, Ba +
Sr und von Ca +Sr sind in Fig. 20 und die der Kombination von
Mg + Ba, Mg + Ca und von Ba + Ca in Fig. 21 wiedergegeben.

Aus Fig. 20 ist zu entnehmen, dass das System Ba +Sr in seinem
Mischverhältnis von 90 ccm $BaCl_2$ + 10 ccm $SrCl_2$ die O_2-Aufnahme der
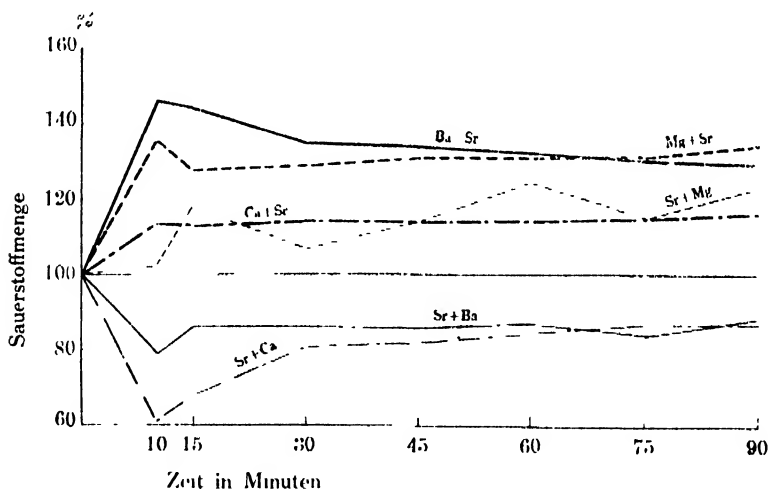
Fig. 20.   Wirkung gemischter Salzlösungen von Mg u. Sr, Ba u. Sr, u. Ca u. Sr

Algen auffallend beschleunigt im Gegensatz zum umgekehrten Misch-
verhältnis (90 ccm $SrCl_2$ + 10 ccm $BaCl_2$), wo sie erheblich herabgesetzt
und zwar viel kleiner als die Norm ist.   Man ist also berechtigt, daraus

zu schliessen, dass die Wirkung des Ba durch Zusatz einer kleinen
Menge Sr weiter beschleunigt und im anderen Falle die Wirkung des
Sr in Gegenwart einer kleinen Menge Ba stark gehemmt wird.

Mit etwas kleineren Ausschlägen als beim System Ba + Sr verhält
sich auch das System Ca + Sr ähnlich. Zwar wirkt es in seinem
Mischverhältnis 90 ccm CaCl$_2$ + 10 ccm SrCl$_2$ anregend auf die O$_2$-
Absorption, indem die Kurve ganz ähnlich der verläuft, die bei der
reinen SrCl$_2$-Lösung vorliegt. Dagegen ist beim Gemisch von 90 ccm
SrCl$_2$ + 10 ccm CaCl$_2$ charakteristisch, dass die Kurve in den ersten
10 Minuten ihr Minimum erreicht und sich bis zum Abschluss des
Versuchs in viel niedrigerer Höhe als die der Kontrolle hält.

Das gleiche Verhältnis kann man auch beim System Mg + Sr wahr-
nehmen. Und zwar beschleunigt ein kleiner Zusatz von Sr die O$_2$-
Aufnahme stark, während durch die Gegenwart einer kleinen Menge
Mg im umgekehrten Mischverhältnis die beschleunigend Wirkung von
Sr besonders in den ersten 10 Minuten gehemmt wird.

Wie man aus Fig. 21 sehen kann, wirkt beim System Mg + Ba
ein kleiner Zusatz von Mg (90 ccm BaCl$_2$ + 10 ccm MgCl$_2$) ebenfalls
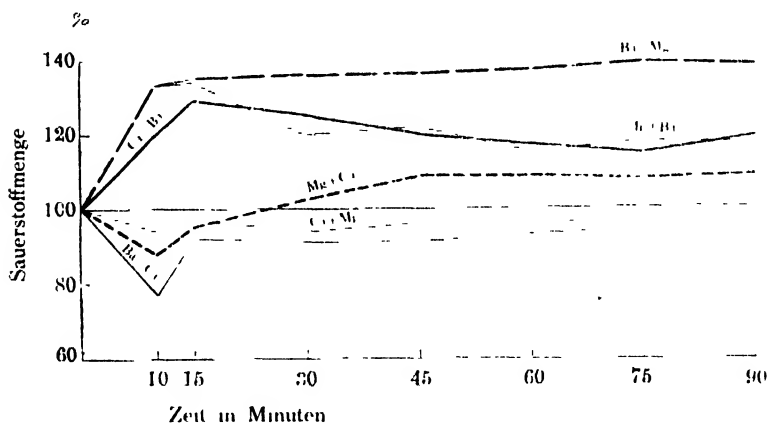
Fig. 21. Wirkung gemischter Salzlösungen von Mg u. Ba, Mg u. Ca, u. Ba u. Ca

stark beschleunigend, im umgekehrten Mischverhältnis dagegen die
Gegenwart einer kleinen Menge Ba nach anfänglicher Beschleunigung
hemmend, sodass sich die Kurve nach Ablauf von 90 Minuten der
der reinen Mg-Lösung nähert.

Ähnliche Verhältnisse sind auch bei den Systemen Mg + Ca und Ba + Ca zu beobachten.

So sind auch bei den zweiwertigen Kationen die gleichen Verhältnisse wie bei den einwertigen wahrzunehmen. Und zwar entscheidet die Stellung eines Kations in der Reihe Sr$<$Mg$<$Ba$<$Ca darüber, ob ein Kation reizend oder hemmend wirkt. Der Abstand zwischen je zwei Kationen in der Reihe ist dabei im grossen und ganzen ausschlaggebend dafür, wie stark sie aufeinander wirksam sind.

VII. Diskussion.

Es ist seit langem bekannt, dass reine Salzlösungen auf verschiedene Lebenserscheinungen schädlich wirken, indem sie sich je nach der Art und der Konzentration fördernd oder hemmend äussern. So ist z. B. die beschleunigende Wirkung von Na- und K-Salzen, jedenfalls in sehr verdünnter Lösung, auf die Atmung von Jacobi (1899), Kosinski (1902) Brooks (1920), Gustafson (1920), Lyon (1921), Lundegårdh (1924) und Tamiya (1928) bei verschiedenen Pflanzen festgestellt worden.

In meinem Versuche an *Chlorella* habe ich beobachtet, dass der O_2-Verbrauch in der 1 m-NaCl-Lösung geringer als in der Norm ist, aber von der 10^{-1} m-Verdünnung an stark beschleunigt wird. Die Ursache dafür, dass Lyon (1921) bei *Elodea canadensis* eine geringere CO_2-Ausscheidung in der 10^{-1} m- NaCl-Lösung als in der Norm beobachten konnte, ist wahrscheinlich darin zu suchen, dass *Elodea canadensis* gegen mechanische oder chemische Reize weniger widerstandsfähig als unsere Versuchspflanze war. Es muss hier betont werden, dass überall in meinem Versuche beim Vergleich der hier gewonnenen Ergebnisse mit anderen die Berücksichtigung dieser Tatsache wichtig war.

Was nun Li und Rb anbetrifft, so ist auch von Benecke (1907), Ono (1900) Cholodonyj (1923) und anderen die günstige Wirkung auf das Wachstum und die Plasmabewegung der verschiedenen Pflanzen nachgewiesen. u. a. hat Cholodonyj (1923) den Einfluss des Rb-Ions auf den Geotropismus der Wurzel von *Lupinus albus, Zea mays* u. a. studiert und nachgewiesen, dass das Rb-Ion eine etwas geringere Giftigkeit als Li besitzt. Bei *Chlorella* übt aber Rb eine stärkere Wirkung auf die Atmung aus, und erst in der 10^{-1} m-Verdünnung

wird der O_2-Verbrauch etwas grösser als der in der Nährlösung.

Schädliche Einflüsse der Erdalkalisalze, ähnlich den eben geschilderten, sind auch bei verschiedenen Pflanzen von GÜNTHER (1897), TAMIYA (1928), BROOKS (1920) CHOLODONYJ (1923) u. a. konstatiert worden. So gibt z. B. LYON (1921) an, dass in einer 0,07 m- CaCl-Lösung die von *Elodea canadensis* nach Ablauf von 90 Minuten verbrauchte O_2-Menge nur etwa 60% der Norm betrug. Man sieht, dass diese Zahl doch viel grösser als die bei *Chlorella* ist, weil sie dort selbst in einer 10^{-2} m- Lösung kaum mehr als die Hälfte der oben genannten Zahlen betrug. Solch schädliche Wirkung wie die des Ca-Ions auf den O_2-Verbrauch von *Chlorella* konnte ich in den Alkali- und den übrigen Erdalkalikationen nicht feststellen. Hiernach sollte man annehmen, dass diese Ergebnisse auf der Beseitigung der Ca-Salze aus der Nährlösung, wie das bei meinen Versuchen geschehen ist, beruhen; trotzdem ist von mehreren Forschern (MOLISCH, BENECKE) nachgewiesen worden, dass die Ca-Ionen für die Kultur der grünnen Algen überflüssig sind. Man kann also diese Resultate als die eigentlich physiologischen Wirkungen des Ca-Ions auf den Sauerstoffverbrauch dieser Algen betrachten.

Was zunächst den Mechanismus der toxischen Wirkung reiner Salzlösungen anbelangt, so hat HANSTEEN-CRANNER (1910-191 t) durch seine Arbeiten viel zur Klärung dieser Frage beigetragen. Er fand durch zahlreiche Untersuchungen die wichtige Tatsache, dass die Ursache der Giftigkeit der Salze nicht in der Strukturveränderung im Innern der Zelle liegt, sondern in den Oberflächenwirkungen zu suchen ist. Es wurden in erster Linie nur solche Pflanzenorgane angegriffen, die in unmittelbaren Kontakt mit der schädlichen Lösung geraten und bei denen ein schnelles Flächenwachstum der jungen Zellen vor sich geht; die von der Flüssigkeit nicht berührten Gewebeteile bleiben, auch wenn sie den angegriffenen direkt benachbart sind, intakt.

Zur Erklärung der Giftigkeit reiner Salzlösung genügt es nicht allein, ihre kolloidchemische Wirkung auf die Zellwand zu berücksichtigen. Einige Salze üben schon nach kurzer Zeit auf erwachsene Zellen eine Giftwirkung aus, wobei die Zellwand ihr normales Aussehen behält. Bekanntlich lässt die Zellwand einer lebenstätigen Zelle die Salzlösung fast momentan durch, und die Wirkung der letzteren richtet

sich hauptsächlich auf die Oberfläche des Plasmas, was dann zunächst
dem weiteren Eindringen der Salze Widerstand leistet. Bald aber
dringen die Salze doch in das Plasma ein und zwar mit sehr verschie-
dener Geschwindigkeit. Dringt nun das Plasmolyticum in die Vakuole
ein, so wird das Plasma wieder an die Zellwand gepresst, und aus
der Geschwindigkeit dieses Prozesses kann man auf das Eindringen
der Lösung Schlüsse ziehen. Um zu zeigen, welcher Zusammenhang
zwischen der Permeabilität des Plasmas gegenüber einem Stoff und
seiner Giftigkeit besteht, soll nun das Eindringungsvermögen der Alkali-
und Erdalkalikationen untersucht werden.

Für das Eindringungsvermögen der Salze verschiedener Kationen
gibt TRÖNDLE (1918) folgende Reihe (vorwiegend Chloride): $Ca<$
$Sr<Ba<Mg<Li<Na<K<Rb$, d. h. die Alkalisalze dringen viel
leichter als die Erdalkalien in das Plasma ein. Durch die Methode
der Grenzplasmolyse verglich auch FITTING (1915 1917) die Anfangs-
geschwindigkeit des Grenzplasmolyseneintritts verschiedener Alkalisalze
und stellte fest, dass, wenn man die Kationen variiert, sich die Reihe:
$Li<Na<K$ ergibt. Das Vermögen der Kationen, das Wachstum der
Helianthus-Keimlinge zu unterdrücken, nimmt nach BOROWIKOW (1916)
folgender Reihe entsprechend zu: $NH_4<K<Na<Li<Mg<Ba<$
$Ca<Sr<Ni<Mn- Hg<Cu$. Vor BOROWIKOW fand schon SARAN-
DINASKI (1912) für das Wachstum junger Keimlinge folgende Hem-
mungsreihe (neutrale Lösung, Salze 0.001 m, Chloride): $NH_4<K<$
$Na<Li<Mg<Ca<Sr<Ba<Ni<Mn<Cu<Hg$. Diese beiden stim-
men gut miteinander überein, indem sich die Kationen in beiden
Reihen nach den Gruppen der steigenden kolloidchemischen Wirksam-
keit ordnen.

BOAS (1921) untersuchte den Einfluss der Salze auf die Hefegärung
der wichtigen Zuckerarten und fand, dass die Salze die Gärungsinten-
sität den folgenden Reihen entsprechend fördern: $Li<Na<NH_4<$
$K, Mg<Ca<Ba$. Dass bei der giftigen Wirkung der Salze deren
Eindringen ins Plasma eine Hauptrolle spielt, folgt aus den Unter-
suchungen von SPEK (1921-1923) und KAHO (1921 -'26). Beide Au-
toren kommen zu dem Schluss, dass die Giftigkeit der Neutralsalze
parallel mit der Permeabilität des Plasmas für diese Salze zunimmt.
Nach SPEK sollen ausserdem diejenigen Salze, welche besser eindringen,
auch die Wasseraufnahme durch das Plasma begünstigen, während sie

durch die schlecht eindringenden Salze gehemmt wird.

Nach meinen Versuchen hemmen Alkali- und Erdalkalikationen den Sauerstoffverbrauch dieser Algen nach folgender Reihe:

Alkalikationen: K$<$Na$<$Li$<$Rb.
Erdalkalikationen: Sr$<$Mg$<$Ba$<$Ca.

Im Vergleich mit der Reihe Boas' weicht die Stellung des Magnesiums bei mir von der bei ihm ab. Diese Tatsache spricht in stärkster Weise dafür, dass die schädliche bzw. stimulierende Wirkung der Alkali- und Erdalkalikationen in grossen und ganzen mit der Reihenfolge der Permeabilität übereinstimmt. Natürlich ist die Fähigkeit der Neutralsalze, in das Plasma einzudringen, ein physikochemischer Prozess, der im Zusammenhang mit dem Vermögen der Salze, den kolloidalen Zustand des Plasmas zu ändern, steht. Und diese Wirkungen sind in erster Linie auf reversible Zustandsänderung des Plasmas zurückzuführen.

Früher hat HUEPPE (1896) Folgendes als „ biologisches Grundgesetz " formuliert: Jeder Körper, der in bestimmter Konzentration Protoplasma tötet und vernichtet, hebt in geringerer Menge die Entwicklungsfähigkeit auf, wirkt aber in noch geringerer Menge, jenseits eines Indifferenzpunktes, umgekehrt als Reiz und erhöht die Lebenseigenschaften. Nach meinen Versuchsergebnissen trifft dies auch beim Sauerstoffverbrauch von *Chlorella* zu.

Was endlich die antagonistische Ionenwirkung anbetrifft, so wird sie durch die gegenseitige Verdrängung der verschiedenen Ionen bei der Adsorption erklärt, weil nach MASIUS (1908) alle Einzelstoffe im Gemische schwächer als in reinen Salzlösungen adsorbiert werden. Einzelne stärker adsorbierbare Stoffe werden auch im Gemische stärker adsorbiert, Ionen mehrwertiger Metalle werden stärker adsorbiert als Ionen einwertiger, sodass es verständlich ist, weshalb eine verhältnismässig kleine Menge von Calciumsalzen eine grössere Menge von Natriumsalzen entgiften kann.

Werfen wir noch einmal die Frage auf, inwiefern bei physiologischem Ionenantagonismus die Adsorptionserscheinung eine Rolle spielt. Man findet in der Literatur sehr häufig Hinweise auf die gegenseitige Ionenverdrängung bei antagonistischen Erscheinungen, wobei die stär-

ker adsorbierenden Ionen die schwächer adsorbierenden verdrängen. Jedoch durch Adsorption allein können wir die antagonistische Kationenwirkung nicht erklären ; denn es bleibt dann vollständig unverständlich, warum die Ionenreihen mit den Reihen der Adsorption und der Giftwirkung nicht immer übereinstimmen. Daraus geht hervor, dass die Adsorptionserscheinungen beim Ionenantagonismus keine dominierende Rolle spielen können.

KAHO (1921) untersuchte die gegenseitige Hemmung der Aufnahme in Salzgemischen näher und kam zu folgenden Ergebnissen: Die antitoxische Wirkung eines Salzes steht im engsten Zusammenhang mit seiner permeabilitätshemmenden Wirksamkeit; je mehr ein Neutralsalz das Eindringungsvermögen eines anderen (giftigen) Salzes herabsetzt, desto stärker wirkt es antitoxisch ; die permeabilitätshemmende Aktivität eines Salzes ist seiner Eindringungfähigkeit umgekehrt proportional, je schwerer ein Salz selbst in das Plasma eindringt, um so grösser ist seine antagonistische Wirkung den anderen Salzen gegenüber.

Für Kationen, die sich in Gemischen zweier Salze mit demselben Anion befinden, ergaben sich folgende praktische Regeln : Die Aufnahme eines jeden Salzes der Permeabilitätsreihe K$>$Na$>$Li$>$Mg$>$Ba$>$Ca wird durch ein beliebiges anderes Salz gehemmt, dessen Kation in der Reihe rechts von dem ersten steht. Diese Hemmung ist um so stärker, je grösser der Abstand zwischen den Kationen in der Reihenfolge ist. Die zweiwertigen Kationen haben ihrer grösseren Kolloidaktivität gemäss eine bedeutend grössere Hemmungskraft. Durch exakte Versuche bestätigte MACDOUGAL, dass, wie schon KAHO berichtet hatte, beim Permeieren die Kolloidaktivität der Salze massgebend ist. Wenn die den O_2-Verbrauch beschleunigende Kationenreihe K$>$Na$>$Li$>$Rb bzw. Sr$>$Mg$>$Ba$>$Ca bei *Chlorella* der Permeabilitätsreihe der betreffenden Kationen gleich gestellt werden kann, so steht die genannte Regel auch mit unseren Ergebnissen im Einklang, wie wir im vorigen Abschnitte beim physiologischen Antagonismus der ein- oder zweiwertigen Kationen gesehen haben.

Anderseits kann man etwa aus den Lecithinversuchen von NEUSCHLOSS (1920-1921) ersehen, dass der Kationenantagonismus sich nicht bloss bei einem bestimmten Verhältnis je einer absoluten Ionenkonzentration äussert, sondern, dass unabhängig von der absoluten Konzen-

tration das Konzentrationsverhältnis zweier Ionen allein entscheidet. Diese Erscheinung wird auch durch die Versuche von LOEB (1915) an *Balanus*-Larven bestätigt. Sie eignen sich für solche Versuche, ähnlich wie *Fundulus*-Arten, deswegen, weil ihr Leben weitgehend von dem osmotischen Druck ihrer Umgebung unabhängig ist. LOEB fand nun, dass die Larven nur bei einem einigermassen bestimmten Konzentrationsverhältnis zwischen Na : K einerseits und Ca : Mg anderseits, nämlich im Mittel 35 : 1, normal umherschwimmen, wenn auch die absolute Konzentration um etwa das zehnfache variierte.

Die Abhängigkeit der antagonistischen Salzwirkung von dem Mischverhältnis zweier Lösungen tritt auch bei meinen Versuchen hervor. So konnten wir z. B. im Gemisch von Na : K 1 : 9, wie Fig. 18 zeigt, fast keinen Antagonismus, vielmehr beschleunigende Wirkung auf den Sauerstoffverbrauch von *Chlorella* bemerken, während er im Gemisch des umgekehrten Verhältnisses, d. h. Na : K = 9 : 1, deutlich war.

Die Hypothese PFEFFER's betr. der Existenz einer Plasmamembran brachten auch HÖBER und LOEB (1916) zum Ausdruck. Der zuletzt genannte Autor nimmt an, dass reines NaCl die Kolloide der Plasmaoberfläche erweicht und sie permeabel macht, während mehrwertige Ionen ihre Verhärtung verursachen. Der zuerst genannte Autor erklärt die antagonistische Salzwirkung als eine Entquellung der Plasmamembran durch die zweiwertigen Ionen. Auch OSTERHOUT (1905) führt die antagonistische Salzwirkung auf eine Permeabilitätsänderung der Protoplasmaoberfläche zurück. Er zeigte, dass die Plasmolyse der Pflanzenzelle durch reine Lösungen von Natriumchlorid oder Calciumchlorid einer grösseren Salzkonzentration bedürfe als die Plasmolyse durch das Gemisch dieser Salze, welches denselben osmotischen Druck hat. Daraus schloss er, dass beide Salze sich gegenseitig am Eintritt in die Zelle hindern. Anderseits vergrössert sich nach OSTERHOUT die elektrische Leitfähigkeit der Gewebe von *Laminaria* nach dem Überführen aus Seewasser in reine Natriumchloridlösung bis zur Leitfähigkeit der toten Gewebe. Calciumsalze rufen zunächst eine Verminderung der Leitfähigkeit und erst später eine solche Erhöhung hervor, wie sie Natriumsalze verursachen. Wenn aber in der Lösung beide Salze vorhanden sind, so nehme die Leitfähigkeit des lebenden Gewebes zuerst ab, um alsdann langsam zu zunehmen. Die geringe

Leitfähigkeit der lebenden *Laminaria*-Gewebe schreibt OSTERHOUT der geringen Permeabilität des Protoplasmas für Salze, die Wirkung der einzelnen Salze ihrer Vergrösserung oder Verminderung zu. Im Gemisch sollen dagegen die beiden Salze sich gegenseitig am Eintritt in die Zelle hindern. Die Versuchsmethodik OSTERHOUT's scheint aber nach HÖBER für die Erklärung der Verhältnisse in lebenden Zellen nicht passend zu sein, weil sich die Gewebe in den Versuchen OSTER-HOUT's unter anomalen Bedingungen befanden. Dass aber dabei die Permeabilitätsveränderung die Hauptrolle spielen, ist bis jetzt nicht bewiesen. Im Gegenteil ist es nicht ausgeschlossen, dass die antagonistische Salzwirkung sich im Zellinnern abspielt, wie z. B. Versuche von REED (1918) zeigten.

Man kann also zur Zeit noch kein vollständiges Bild der antagonistischen Ionenwirkung auf das Protoplasma entwerfen; man darf aber voraussetzen, dass sich durch gegenseitige Verdrängung der Ionen bei der Permeabilität die antagonistischen Erscheinungen erklären lassen.

VIII.   ZUSAMMENFASSUNG.

Die Resultate der vorstehenden Untersuchungen lassen sich, wie folgt, zusammenfassen :

1. *Chlorella* braucht viel mehr Sauerstoff im doppeldestill. Wasser als in Nährlösung. Natürlich kann dies eine temporäre Erscheinung sein, doch hält dieser Zustand wenigstens 1½ Stunde lang an.

2. In Nährlösung beträgt die durch 0.1 ccm (frisches Volumen) *Chlorella* verbrauchte Sauerstoffmenge während 90 Minuten ca. 160–200 cmm.

3. In bezug auf den O_2-Verbrauch von *Chlorella* kommt im allgemeinen den einzelnen konzentrierten Alkalikationen ein schädlicher Einfluss zu, während verdünnte Alkalikationen erregend wirken. Unter sämtlichen Alkalikationen sind Rb am meisten und die K-Ionen am wenigsten schädlich. Die Reihenfolge der beschleunigenden Kationenwirkung auf den Sauerstoffverbrauch ist je nach der Konzentration verschieden, aber man kann die folgende in ihrer Schädlichkeit steigende Reihe als gültig annehmen : K<Na<Li<Rb.

4. Ähnlich wie bei den vorigen liegt der Fall bei den Erdalkalikationen, u. zwar kommt jedem einzelnen konzentrierten Erdalkalikation

ein schädlicher und im Vergleich zum verdünnten beschleunigender Einfluss zu. Ca ist das schädlichste und Sr das günstigste. Die Wirksamkeit der einzelnen Kationen ist je nach der Konzentration verschieden, aber die Reihenfolge der Wirksamkeit kann etwa, wie folgt, aufgestellt werden: $Sr < Mg < Ba < Ca$.

5. Bei der O_2-Aufnahme durch *Chlorella* sind antagonistisch die zweiwertigen gegen die einwertigen Kationen etwa in folgenden Reihenfolgen wirksam:

$$Bei\ Ca: \quad Rb < Na < K < Li$$
$$Bei\ Ba: \quad Li < Rb < Na < K$$
$$Bei\ Mg: \quad Rb < Li < Na < K$$
$$Bei\ Sr: \quad Li < Rb < Na < K$$

6. Die antagonistischen Wirkungen eines Salzes gegen die anderen ist nicht bloss von der absoluten Konzentration, sondern auch von dem Verhältnis zweier Salzlösungen abhängig.

7. Die Wirkung der ein- bzw. zweiwertigen Kationen untereinander sind ebenfalls aus der Kolloidaktivität der betreffenden Ionen verständlich: Die Kationenreihe $K < Na < Li < Rb$ bzw. $Sr < Mg < Ba < Ca$ ist dabei im grossen und ganzen ausschlaggebend für die Wirkungsweise, indem das Kation links in der Reihe beschleunigend, das rechts hemmend wirkt.

8. Bei dem Sauerstoffverbrauch dieser Algen spielt der Kalkfaktor eine grosse Rolle.

LITERATURVERZEICHNIS

BENECKE, W. 1907: Über die Giftwirkung verschiedener Salze auf *Spirogyra* und ihre Entgiftung durch Calciumsalze. Ber. Deut. Bot. Ges., Bd 25, S 322-337.

BOAS, F 1921. Untersuchungen über die Mitwirkung der Lipoide beim Stoffaustausch der pflanzlichen Zellen Biochem Z, Bd. 117, S. 166-211

BOAS, F 1921: Beiträge zur Kenntnis der Wirkung des Saponins auf die pflanzlichen Zellen. Ber. Deut. Bot. Ges., Bd 78, S 350-353

BÖHM, J. 1875: Sitzungber. d. Kais Akad d Wiss Wien, Bd 71, Abt 1, zit nach HOBER

BOROWIKOW, G 1916 Über die Einwirkung von salzartigen Stoffe auf die Wachstumsgeschwindigkeit der Pflanzen Zitiert nach H KANO 1926 Ergeb d Biol, Bd. 1, S 392.

BROOKS M. M. 1920: Comparative studies on respiration. X Toxic and antagonistic effects of magnesium in relation to the respiration of *Bacillus subtilis*. Jour gen. Physiol, Vol 2, p 331-336

BROOKS, M. M. 1920: Comparative studies on respiration. VIII. The respiration of *Bacillus subtilis* in relation to antagonismus. Ibid., Vol. 2, p. 5-16.

BROOKS, M. M. 1921: Comparative studies on respiration. XIII. Antagonistic action of lanthanum as related to respiration. Ibid., Vol. 3, p. 337-342.

BROOKS, M. M. 1922: The effect of hydrogen ion concentration on the production of carbon dioxide by *Bacillus butyricum* and *Bacillus subtilis*. Ibid., Vol. 4, p. 177-186.

BROOKS, M. M. 1922: The penetration of cations into living cells Ibid., Vol 4, p. 347-349.

CHOLODNYJ, 1923: Zur Frage uber die Beeinflussung des Protoplasmas durch mono- und bivalente Metallionen Beih. z Bot. Centralbl., Bd. 39, S. 231-238.

CHOLODNYJ, 1923: Über den Einfluss der Metallionen auf den Geotropismus der Wurzeln Ibid., Bd 39, S. 239-256.

COOK, S. F. 1926: The rôle of certain metallic ion as oxidation catalysts Jour. gen. Physiol., Vol. 10, p. 289-312

COOKS, S. F. 1926: The effects of certain heavy metals on respiration Ibid., Vol 9, p. 575-601

COOKS, S. F. 1926 A latent period in the action of copper on respiration Ibid., Vol. 9, p. 631-650.

COOKS, S. F. 1926: The toxic action of copper on *Nitella*. Ibid., Vol. 9, p 735-751.

EMERSON, R 1927. The effect of certain respiratory inhibitors on the respiration of *Chlorella*. Ibid Vol. 10. p. 469-477.

FITTING, H. 1915: Untersuchungen über die Aufnahme von Salzen in die lebende Zelle Jahrb f. wiss. Bot., Bd. 56, S. 1-64

FITTING, H. 1917: Untersuchungen über isotonische Koeffizienten und ihren Nutzen für Permeabilitatsbestimmungen. Ibid., Bd. 57, S. 553-612

GENEVOIS, L 1927: Über Atmung und Gärung in grünen Pflanzen. Biochem Z, Bd. 186, S. 461-473

GÜNTHER, H. 1897. Beitr. z mineral Nahrung d. Pilze. Diss Erlangen Zit nach H. TAMIYA.

GUSTAFSON, F. G. 1920. Comparative studies on respiration IX The effects of antagonistic salts on the respiration of *Aspergillus niger*. Jour. gen. Physiol., Vol. 2, p 17 24

GUSTAFSON, F G 1920: Comparative studies on respiration. The effect of hydrogen ion concentration on the respiration of *Penicillium chrysogenum*. Ibid., Vol. 2, p. 617-626

GUSTAFSON, F. G 1921: Comparative studies on respiration. XII. A comparison of the production of carbon dioxide by *Penicillium* und by a solution of dextrose and hydrogenperoxide. Ibid., Vol. 3, p. 35-39.

HANSTEEN-CRANNER, B. 1910: Über das Verhalten der Kulturpflanzen zu den Bodensalzen. I und II. Jahrb. f. wiss. Bot., Bd. 47, S. 289.

HANSTEEN-CRANNER, B. 1914: Über das Verhalten der Kulturpflanzen zu den Bodensalzen. III. Beiträge zur Biochemie und Physiologie der Zellwand lebender Zelle. Jahrb. f. wiss. Bot., Bd. 53, S. 536-598.

HUEPPE. 1896: Naturwissenschaftliche Einführung in die Bakteriologie. Wiesbaden. S.

55. Zitiert nach N. Ono. 1900: Jour. Coll. Sci., Tokyo Imp Univ., Vol. 13, pp. 141–185.

Inman, O. L 1921: Comparative studies on respiration. XVI. Effects of hypotonic and hypertonic solution on respiration. Jour. gen Physiol., Vol. 3, p 533–537.

Inman, O. L. 1921: Comparative studies on respiration XVII Decreased respiration and recovery. Ibid., Vol. 3, p. 663–666.

Iwanoff, N. 1911: Die Wirkung der nützlichen und schädlichen Stimulatoren auf die Atmung der lebenden und abgetöteten Pflanzen. Biochem. Z., Bd 32, S. 74–96.

Jacobi, B. 1899: Über den Einfluss verschiedener Substanzen auf die Atmung und Assimilation submerser Pflanzen. Flora, Bd. 86, S. 289–327.

Kaho, H. 1921: Über die Beeinflussung der Hitzkoagulation des Pflanzenplasmas durch Neutralsalze. I. Biochem. Z., Bd. 117, S. 87–95.

Kaho, H. 1921: Zur Kenntnis der Neutralsalzwirkung auf das Pflanzenplasma. II Ibid., Bd. 120, S. 125–141.

Kaho, H. 1921: Ein Beitrag zur Permeabilität des Pflanzenplasmas für die Neutralsalze. Ibid, Bd. 123, S. 284–303.

Kaho, H. 1926: Das Verhalten der Pflanzenzelle gegen Salze. Ergebn d Biol., Bd. 1, S. 380–406.

Kosinski, I. 1902: Die Atmung bei Hungerzuständen und unter Einwirkung von mecha nischen und chemischen Reizmitteln bei *Aspergillus niger* Jahrb f. wiss Bot., Bd 39, S. 137–204.

Kostytschew, S. und A. Scheloumow. 1912: Über die Einwirkung der Gärungsprodukte und der Phosphate auf die Pflanzenatmung Jahrb. f. wiss Bot., Bd. 50, S. 157–199

Kostytschew, S. 1924: Pflanzenatmung. Berlin. Julius Springer.

Lipman, C B. 1909: Toxic and antagonistic effects of salts as related to ammonifikation by *Bacillus subtilis*. Bot Gaz, Vol 48, p 105 125.

Lipman, C B. 1910: On the lack of antagonism between calcium versus magnesium and also between calcium versus sodium. Ibid., Vol. 49, p 41–50.

Loeb J 1901: Über den Einfluss der Wertigkeit und möglicher Weise der elektrischen Ladung von Ionen auf ihre antitoxische Wirkung Pflüger's Arch. f d gesamt. Physiol., Bd. 88, S. 68–78

Loeb, J. 1902: Studies on the physiological effects of the valency and possibly the electrical change of ions. I. The toxic and antitoxic effects of ions as a function of their valency and possibly electrical change. Amerie Jour Physiol., Vol. 6, p. 411–433.

Loeb, J 1916: The mechanism of the diffusion of electrolytes through the membrane of living cells. III. The analogy of the mechanism of the diffusion for acids and potassium salts. Jour. Biol. Chem., Vol. 27, p. 363 375.

Loeb, J. und Wasteneys. 1911: Die Entgiftung von Kaliumsalzen durch Natriumsalze. Biochem Z., Bd. 31, S. 450–477.

Loeb, J. und Wasteneys. 1911: Die Erhöhung der Giftwirkung von KCl durch niedrige Konzentration von NaCl. Ibid., Bd. 32, S. 150–163.

Loeb, J. und Wasteneys. 1911: Die Entgiftung von Natriumchlorid durch Kaliumchlorid. Ibid., Bd. 33, S. 480–488.

LOEB, J. 1922: Sodium chloride and selective diffusion in living organism. Jour. gen. Physiol., Vol. 5, p. 231–254

LOEB, J. and WASTENESYS. 1915: Proceed. of the nat. Acad. Vol. I. p. 439. Zit. nach HÖBER, R. 1924: Physikalische Chemie der Zelle und der Gewebe. S. 667.

LUNDEGÅRDH, H. und MORAVEK. 1924: Untersuchungen über die Salzaufnahme. I. Mitt. Die gegenseitige Beeinflussung der Ionen Biochem Z., Bd. 151, S 296–309.

LUNDEGÅRDH, H 1924: Der Einfluss der Wasserstoffionenkonzentration in Gegenwart von Salzen auf das Wachstum von *Gibberella saubinutii*. Biochem. Z., Bd. 146, S. 564 572.

LYON, C J 1921: Comparative studies on respiration XVIII. Respiration and antago nism in *Elodea*. Amer. Joun. Bot., Vol. 8, p. 458–463

MACDOUGAL, D. T. 1923: Permeability and the increase of volume of contents of living and of artificial cells. Proc Amer Philosoph. Soc, Vol 62, No 2, p 1-25.

MASIUS. 1908. Über die Adsorption in Gemischen Diss. Leipzig. Zitiert nach Wo. OSTWALD

MAYER, ADOLF 1869 Untersuchungen über alkoholische Garung Zitiert nach JACOBI, B

MAYER, ANDRE et PLANTEFOL, L 1926· Influence des Elektrolytes sur la Respiration des Mousses Ann Physiol et Physicochim Biol., Tom 2

MAYER, ANDRE et PLANTEFOL, L. 1925. Hydratation et respiration chez les Mousses Ibid, Tom I

MOLISCH, H. 1895: Die Ernahrung der Algen (Susswasseralgen, I Abhand.) Sitzungsb. d. k Akad d Wiss Wien, Bd 104, Abt 1, S 383–400

NEUSCHLOSS, S. M. 1920 Die kolloidchemische Bedeutung des physiologischen Ionen-antagonismus und der äquilibrierten Salzlosung PFLÜGER's Arch f d. gesamt Physiol, Bd 181, S 17 39.

NEUSCHLOSS, S. M. 1920. Über die Bedeutung des Ionenantagonismus fur die Hamolyse Ibid., S. 40–41.

NEUSCHLOSS, S. M. 1920 Untersuchungen uber den Einfluss der Neutralsalze auf die Fermentwirkung. Ibid, S. 45–64

NEUSCHLOSS, S M. 1921: Erwiderung auf die Bemerkung des Herren H. HANDOVSKY zu meiner Arbeit: Die kolloidchemische Bedeutung des physiologischen Ionenantagonismus usw. Ibid., Bd. 187, S. 136 138

ONO, N. 1900: Über die Wachstumsbeschleuning einiger Algen und Pilze durch chemische Reize. Joun Coll. Sci Tokyo Imp. Univ, Vol 13, p 141–185.

OSTERHOUT, W J V. 1905· Extreme toxicity of sodium chloride and its prevention by other salts. Joun Biol. Chem., Vol. I, p. 363–369

OSTERHOUT, W. J. V. 1909: On similarity in the behavior of sodium and potassium Bot. Gaz, 48, p. 98 -104.

OSTERHOUT, W. J. V. 1911: Stetige Änderungen in den Formen von Antagonismus-Kurven. Jahrb. f. wiss. Bot., Bd. 51, S 645–650.

OSTWALD, Wo. 1907. Über die Beziehung zwischen Adsorption und Giftigkeit von Salzlösungen für Süsswassertiere. (*Gammarus*). PFLÜGER's Arch f. d. gesamt. Physiol., Bd. 120, S. 19–30.

OSTWALD, Wo. 1910: Über die Beziehung zwischen Adsorption und Giftigkeit. Kolloid-Z., Bd. 6, S. 297 307.

PALLADIN, W., E. HÜBBENET und M. KORSAKOW. 1911: Über die Wirkung von Methylen-blau auf die Atmung und alkoholische Gärung lebender und abgetöteter Pflanzen. Biochem Z., Bd. 18, S. 151–206.

PALLADIN, W. 1909: Über Prochromogene der pflanzlichen Atmungschromogene Ber. Deut. Bot. Ges., Bd. 27, S. 101–166

PALLADIN, W. 1909: Über die Wirkung von Giften auf die Atmung lebender und ab-getöter Pflanzen. Jahrb. f. wiss. Bot , Bd. 47, S. 131–461

PRIANISCHNIKOW. 1895: Über den Eiweisszerfall bei der Keimung (russ) Zitiert nach ZALESKI und REINHARD. 1910: Biochem Z., Bd. 23, S. 193–214.

REED, H. 1918. Adsorption of sodium and calcium by wheat Bot Gaz Vol 66, p 375–580.

REINHARD, A. 1910: Zur Frage über die Salzwirkung auf die Atmung der Pflanzen. Ber. Deut. Bot. Ges , Bd. 28, S. 451 455

REZNIKOFF, P. 1927: Microurgical studies in cell physiology V. The antagonism of cations in their actions on protoplasm of *Amoeba dubia* Jour gen Physiol , Vol 11, p 221–237.

SAKAMURA, T 1914. Wirkung der Elektrolyten auf die Lebenserscheinungen von *Gonium pectrale* und *Pandrina Morum*. (Japanisch, mit deutschen Résume), Bot Mag. Tokyo, Vol 38, S. 79–93

SARANDINAKI, 1912 Beobachtung über die Einwirkung der Salze auf das Wachstum Mém. de la soc. des naturalistes de la nouvelle Russie Odessa, Tom 38, p. 307 Zitiert nach H. KAHO 1926· Ergeb d Biol , Bd 1, S 380-406

SPIK, J. 1921: Experimentelle Beiträge zur Physiologie der Zellteilung Biol Zentralbl , Bd. 39, S. 23-31.

SPIK, J. 1923 Über den physikalischen Zustand von Plasma und Zelle der *Opalina ranarum* Arch f. Protistenkunde, Bd 16, S. 166 202.

TAMIYA, II. 1928. Studien über die Stoffwechselphysiologie von *Aspergillus oryzae* II. Acta Phytochimica, Vol 1, p 77–213.

TRÖNDLE, A. 1918: Sur la perméabilité du protoplasme vivant pour quelques sels Arch. des sciences phys. et nat , Tom. 45, p 38 et p. 167 Zitiert nach II. KAHO. 1926. Ergeb d Biol., Bd. 1, S. 380–406.

WARBURG, O. 1919 Über die Geschwindigkeit der photochemischen Kohlensäurezer-setzung in lebender Zellen. Biochem. Z., Bd. 100, S 230–270.

WARBURG, O. 1920. Über die Geschwindigkeit der photochemischen Kohlensäurezer-setzung in lebenden Zellen. II. Ibid., Bd. 103, S. 188–217.

WARBURG, O. 1923. Versuche an überlebendem Carcinomgewebe (Methoden) Ibid , Bd. 142, S. 317-333.

WARBURG, O. und E. NEGELEIN. 1922 Über den Energieumsatz bei der Kohlensäure-assimilation. Z. f. physik. Chemie, Bd 102, S 235–266.

ZALESKI, W. und A. REINHARD. 1910: Die Wirkung der Mineralsalze auf die Atmung keimender Samen. Biochem. Z., Bd. 23, S 193–214.

ZALESKI, W. und A. REINHARD. 1910: Zur Frage der Wirkung der Salze auf die At-mung der Pflanzen und auf die Atmungsenzyme Ibid , Bd. 27, S. 450–473.

ZALESKI, W. und A. REINHARD. 1911: Untersuchungen über die Atmung der Pflanzen. Ibid., Bd. 35, S. 228-245.

Report of the Biological Survey of Mutsu Bay.
13.   Echinoidea.

By

Th. Mortensen, Copenhagen.

(With Pl. XIX)

The material of Echinoids from the Mutsu Bay, sent me for examination by Professor Sanji Hōzawa, comprises the following 10 species.

1.   Glyptocidaris crenularis A. Agassiz.
(Pl XIX, Fig. 1, Text-fig. 1)

A Agassiz. Revision of the Echini. 1872–74. p. 487. Pl. VII. a, figs 6, 8, 9 (here named *Phymosoma crenulare*).

Yoshiwara. Japanese Echini. Zoological Magazine Tokyo. XVIII 1906. Pl. VII. figs. 1-6. (here named *Coptosoma crenulare*)

L. Döderlein. Die polyporen Echinoiden von Japan. Zoologischer Anzeiger XXX. 1906. p. 520.

H. L. Clark. Hawaiian and other Pacific Echini. Pedinidae   Echinometridae. Mem Mus. Comp. Zool. XXXIV. 1912. p. 228. Pl. 90 5- 10; 92. 1 11; 106. 1 2

Station 2 (I); 29. VII. 1926; off Asamushi; coll. S. Takatsuki; Spec. No. 1120.

Station 2; 10. VIII. 1927; off Asamushi; coll. S. Takatsuki; Spec. No. 1947.

Station 67; 11. VIII. 1926; off Sumichigai; coll. S. Hōzawa; Spec. No. 1118.

The specimens are rather small, from 11 to 34 mm horizontal diameter, the species being known to grow to a much larger size, at least 75 mm horizontal diameter.

2.   Temnopleurus Hardwickii (Gray).
(Pl. XIX, Fig. 3).   •

A. Agassiz. Revision of the Echini. p. 460, Pl. VIII. a 1–3.

Th. Mortensen. Echinoidea; Danish Expedition to Siam Mem. Ac. Sc Copen-

hague. 7. Ser. I. 1904. p. 61, 65. Pl. VI. 32, 34; VII. 21.

H. L. CLARK. Hawaiian and other Pacific Echini. Pedinidae....Echinometridae;
   p. 312.

Station 41 (II) ; 31. VII. 1926 ; coll. S. S. Soyo Maru, off Okunai.
Spec. No. 1110.

3. Temnotrema sculpta A. AGASSIZ.

TH. MORTENSEN. Echinoidea. Danish Exp. to Siam; p. 84. Pl. I 5–6, 8, 19; II.
   6. (under the name of *Pleurechinus variegatus*).

H. L. CLARK Hawaiian and other Pacific Echini Pedinidae . Echinometridae;
   p 321. Pl. 112. 1–2.

Station 94; 24. VII. 1927; off Tairadate; coll. S. TAKATSUKI.
Spec. No. 1783.
(Together with a young *Strongylocentrotus nudus* (?)).

4. Strongylocentrotus nudus (A. AGASSIZ).
(Pl. XIX, Fig. 2; Text-fig 1).

A. AGASSIZ. Revision of the Echini, p. 448.

I. DÖDERLEIN Die polyporen Echinoiden von Japan. Zool Anz XXX. 1906 p
   518. (under the name of *Strongyloc. hokkaidensis.*).

YOSHIWARA. Japanese Echini, Pl. XII. 1-2.

H. L CLARK. Hawaiian and other Pacific Echini. Pedinidae . Echinometridae;
   p. 363. Pl. 94. 17–23.

Station 26; 29. VIII. 1927. Futagojima ; coll. S. TAKATSUKI ; spec.
No. 2313.

Station 104; 17. VIII. 1927. Sai ; coll. S. HŌZOWA ; spec. No. 2089.

Station 91; 24. VII. 1927 ; off Tairadate ; coll. S. TAKATSUKI.
Spec. No. 1783. (One very young specimen, 6 mm horizontal diameter,
together with *Temnotrema sculpta;* identification not beyond doubt).

Mediumsized specimens of this species (which grows to a large
size, up to ca. 85 mm horizontal diameter) have on account of their
long dark coloured spines a very considerable general likeness with
Anthocidaris crassispina (A. Ag.), the littoral Echinoid so very com-
mon in the more southern parts of Japan, for instance at Misaki ;
quite young specimens again may also bear a great resemblance to
Pseudocentrotus depressus (A. Ag.), likewise a very common littoral
species in the southern parts of Japan, when their spines are brown,

as is the case in young specimens which I have seen. (Whether the
young specimens have always the spines more light coloured I cannot
tell ; the little material available does not allow any definite conclusions
as to this). A careful study of the oral side of the test will lead to
distinguish the three forms, but this requires a good deal of special
knowledge and is by no means an easy task. Fortunately it is possible
to distinguish the three forms much more easily and with full certainty
by means of the spicules of their tubefeet. In *Strongylocentrotus nudus*
(text-fig. 1) the spicules are arc-shaped, with the ends somewhat irre-
gularly branched ; in *Pseudoc. depressus* (text-fig. 2) they are arc-shaped
with bifid ends and with a pair of spines on the outer side; in
Anthocid. crassispina (text-fig. 3) they are " biacerate ", slightly curved
rods with a spine in the middle. It may be added that in *Glyptocid-*
aris crenularis (text-fig. 4) the spicules are straight rods, usually with
some holes in the middle. Further, in *Strongyloc. pulcherrimus* (text-
fig. 5) they are C-shaped ("bihamate"), while in *Strongyloc. inter-*
medius (text-fig. 6) they are in the main like those of *Strongyloc.*
nudus. Thus all these polyporous Echinoids are distinguished very
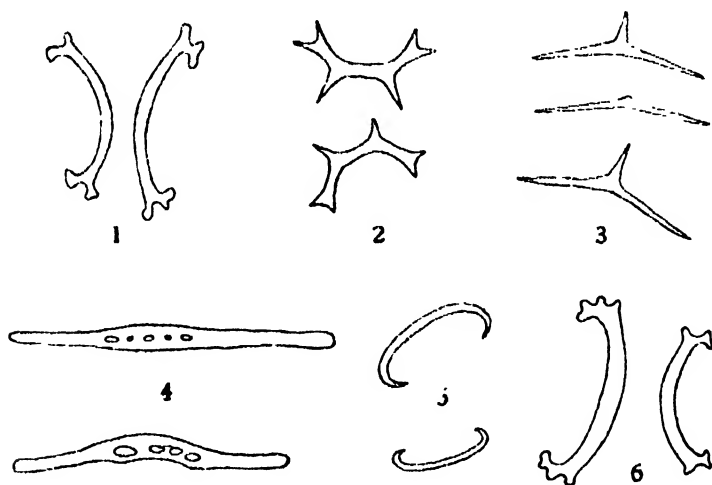
Text-figures 1-6. Spicules from tubefeet of *Strongylocentrotus nudus* (A
AGASSIZ) (1), *Pseudocentrotus depressus* (A. AGASSIZ) (2), *Anthocidaris crassispina*
(A. AGASSIZ) (3), *Glyptocidaris crenularis* A. AGASSIZ (4), *Strongylocentrotus*
pulcherrimus (A. AGASSIZ)(5), and *Strongylocentrotus intermedius* (A. AGASSIZ) (6).
All × 125.

easily, and with full certainty, even when quite young, merely by examining the spicules of their tubefeet. Only *Str. intermedius* and *nudus* cannot be distinguished with certainty by their spicules, but the much shorter and more numerous spines of the former distinguish it very well from *Str. nudus* with its longer and less numerous spines.

5. Strongylocentrotus intermedius (A. AGASSIZ).
(Pl. XIX, Fig. 1; Text-fig. 6)

A. AGASSIZ Revision of the Echini. p. 445.
L. DÖDERLEIN Die polyporen Echinoiden von Japan Zool. Anz. XXX. p. 517.
H. L. CLARK. Hawaiian and other Pacific Echini. Pedinidae .Echinometridae, p. 353.

Station 14; 22. VI. 1927. Futagojima; coll. S. TAKATSUKI; Spec. No. 1596.

Station 103; 14. VIII. 1927. Ôma; coll. S. HÔZAWA. Spec. No. 2065.
Station 104; 18. VIII. 1927. Ôma; coll. S. HÔZAWA; Spec. No. 2079.
Station 105; 18. VIII. 1927. Ôma; coll. S. HÔZAWA; Spec. No. 2190.

The specimens from the three latter stations are very young, only 3-6 mm horizontal diameter; the identification of them as *Str. intermedius* however seems beyond doubt.

6. Strongylocentrotus pulcherrimus (A. AGASSIZ).
(Text-fig. 5)

A. AGASSIZ. Revision of the Echini, p. 453. (under the name of *Sphaerechinus pulcherrimus*).
TH. MORTENSEN. Echinoidea. I. The Danish "Ingolf" Expedition. IV. 1 1903. p. 121. Pl. XX. 10.
YOSHIWARA. Japanese Echini. Pl. XIII. 1-4 (under the name of *Sphaerechinus pulcherrimus*).
L. DÖDERLEIN. Die polyporen Echinoiden von Japan, p. 516
H. L. CLARK. Hawaiian and other Pacific Echini Pedinidae Echinometridae, p. 353.

Station 1; 11. VIII. 1927. Yunoshima: coll. S. HÔZAWA. Spec. No. 1949.

Station 16; 7. VII. 1916. Coast of Tsuchiya; coll. S. HÔZAWA & S. TAKATSUKI. Spec. No. 1119.

Station 101; 19. VIII. 1927. Sai; coll. S. HÔZAWA; Spec. No. 2011.

7. Peronella rubra Döderlein.

I. Döderlein. Seeigel von Japan u. d. Liu-Kiu-Inseln. Arch. f Naturgeschichte. LI. 1885. p. 106.

II. L. Clark. Hawaiian and other Pacific Echini. Clypeastridae. Mem Mus Comp. Zool. 46. 1914. p. 54. Pl. 124. 18–20; 142. 5–7.

Station 103; 17. VIII. 1927. Sai; coll. S. Hôzawa. Spec. No. 2064.

The single specimen at hand is a young one, 18 mm long, with the genital openings not yet developed. The position of the periproct almost halfway between the mouth and the test margin, and the presence of spines on the periproctal plates, however, show rather certainly that it must be the above named species.

8. Echinarachnius mirabilis (A. Agassiz).

A Agassiz. Revision of the Echini, p. 526, Pl. XIII. a 5 6
Yoshiwara. Japanese Echini, Pl. XVI. 9–10.
H. L. Clark Hawaiian and other Pacific Echini. Clypeastridae, p 69, Pl. 125 6

Station 6; 5. VII. 1926. Asamushi; coll. S. Takatsuki. Spec. No. 1130. a.

Station 53 (II); 7. VIII. 1926. Coast of Aburakawa; coll. S. Hôzawa. Spec. No. 1130. b.

9. Echinarachnius griseus Mrtsn.

Th. Mortensen. A new species of the genus Echinarachnius from Japan. Annot Zool. Japonenses. XI. 1927. p. 195. Pl I

Station 23 (I); 16. VIII. 1926. Moura; coll. S. Hôzawa. Spec. No. 1124.

Station 109; 19. VIII. 1927. Fukuura; coll. S. Hôzawa. Spec. No. 2220.

In his Catalogue of the Recent Sea-Urchins in the collection of the British Museum (1925) H. L. Clark states (p. 168) that the young specimens of *Echinarachnius mirabilis* " are remarkable for their very delicate, usually white or very light-coloured tests, while adults are fairly stout and are deep violet in colour." He has, however, found some young specimens as dark as the adult, but he supposes that this is due to these dark specimens being, in spite of their small size,

older than the more light coloured specimens; or the difference in colour "may possibly be correlated with locality and habitat".

Although not having examined the small specimens mentioned by CLARK I cannot help suggesting that the difference in colour between these young specimens is due, not to various age or habitat, but to their representing two different species, the dark ones *Echinarachnius mirabilis*, the light coloured ones *Ech. griseus*. (The latter species had not yet been described when CLARK wrote the work quoted). As for the young specimens from Mutsu Bay there is, at least, no doubt that the dark and light coloured specimens represent two distinct species, the dark ones *Ech. mirabilis*, the light coloured ones *Ech. griseus*.

Besides by their dark colour, which I find to be equally dark in the young and the adult specimens, the young *Ech. mirabilis* also differs from *Ech. griseus* in the usually more marginal position of the periproct; in *Ech. griseus* this is usually more distinctly supramarginal. In general *Ech. mirabilis* is a more robust form than *Ech. griseus*, as also the coat of spines is more dense than in *Ech. griseus*; in the microscopical structure of spines and pedicellariae there is no very tangible difference between the two species. It would appear that in *Ech. griseus* the genital pores are generally formed somewhat earlier than in *Ech. mirabilis*. I have found them already at a size of 10 mm length in *Ech. griseus*, but not until a size of 22 mm in *Ech. mirabilis*; but, on the other hand, they are not formed either in a specimen of 14 mm length of *Ech. griseus*. This accordingly is no reliable difference.

10. Echinocardium cordatum (PENNANT).

A AGASSIZ. Revision of the Echini, p. 319. Pl. XX. 5–7.

TH MORTENSEN. Echinoidea. II. The Danish "Ingolf" Expedition. IV. 2. 1907. p. 145 Pl. XVI. 21; XVII. 15, 21 23, 30, 34, 37–38, 43, 48–49.

H L. CLARK. Hawaiian and other Pacific Echini. Spatangidae. Mem. Mus. Comp. Zool. 46 1917. p 262.

Station 8; 1. VI. 1926. Off Aomori; coll. S. TAKATSUKI. Spec. No. 1113.

Station 17 (I); 17. VII. 1926. Namiuchi; coll. S. HÔZAWA. Spec. No. 1129.

Station 22 (I) ; 20. VII. 1926. Off Moura-Kojima ; coll. S. Hôzawa. Spec. No. 1123.

Station 26 (I) ; 2. VII. 1926. Off Futagojima ; coll. S. Takatsuki. Spec. No. 1122.

Station 27 (I) ; 22. VII. 1926. Off Asamushi; coll. S. Hôzawa. Spec. No. 1126.

Station 30 (I) ; 24. VII. 1927. Off Itanosaki; coll. S. Hôzawa. Spec. No. 1128.

Station 60 ; 9. VIII. 1926. Off Kusodomari ; coll. S. Hôzawa. Spec. No. 1117.

Station 68 ; 2. VIII. 1927. Off Jogasawa ; coll. S. Hôzawa. Spec. No. 1125.

Station 69. (V) ; 11. VIII. 1926. Off Oniuata ; coll. S. Hôzawa. Spec. No. 1121.

Station 107 ; 19. VIII. 1927. Fukuura ; coll. S. Hôzawa. Spec. No. 2208.

Station 98 ; 25. VII. 1927. Noheji ; coll. S. Takatsuki. Spec. No. 1826.

Station 99 ; 25. VII. 1927. Off Asadokora ; coll. S. Takatsuki. Spec. No. 1838.

EXPLANATION OF THE PLATE .

Fig. 1. *Glyptocidaris crenularis* A Agassiz.
Fig. 2. *Strongylocentrotus nudus* (A. Agassiz)
Fig. 3. *Temnopleurus Hardwickii* (Gray.)
Fig. 4. *Strongylocentrotus intermedius* (A. Agassiz.)

(All figures natural size.)
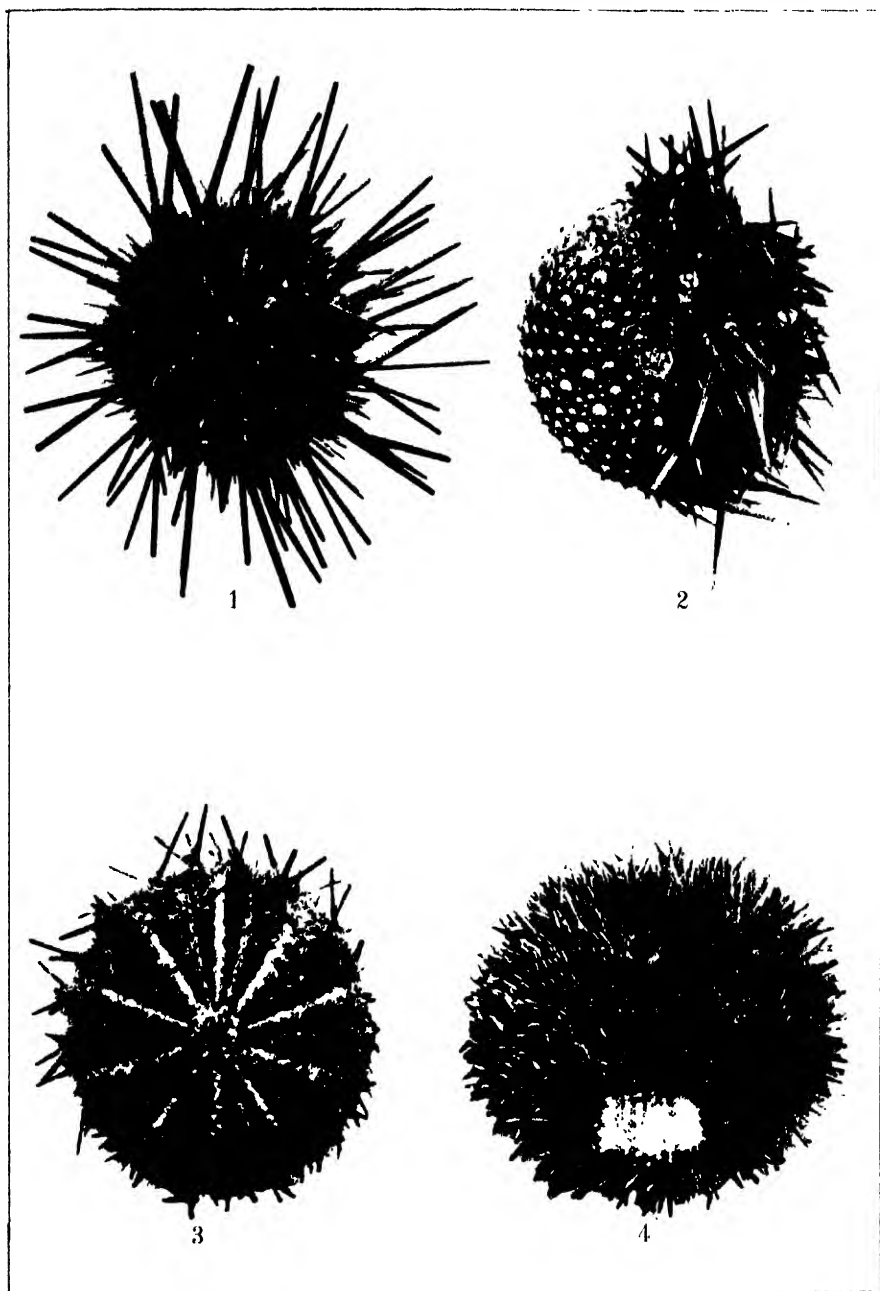
The Fiber Tracts of the Cerebrum of *Carassius auratus* (L.).

By

HIDEOMI TUGE.

(Biological Institute, Tôhoku Imperial University, Sendai)

(With Plates XX, XXI & 33 Text-figures)

I. INTRODUCTION.

Carassius auratus (L.) is one of the commonest fresh water fish living in Japan and can be obtained in large quantities at any season of year. On account of its hardiness under confinement as well as easiness in obtaining the small fry, this fish is becoming one of the favorite materials for various kinds of investigation. The well known " Goldfish " is believed to be a variety of *Carassius auratus* (L.) produced by domestication for a long period, and furthermore *Carassius* itself shows considerable variation according to locality as well as to environment.

Recently another natural variety of this fish called " Tetsugyo " or Ironfish was found at several far isolated districts in Japan, which shows apparently intermediate characters of goldfish on one side and those of *Carassius auratus* (L.) on the other. These three types of fishes which are assumed to be geneticaly closely related to one another suggest many problems of biological interest, and indeed this subject is duly drawing the interest of several workers at this Biological Institute.

I undertook to study the brains of these three forms of fishes with a view to determine whether or not any differences characteristic to each may be found, and as the first step I have focussed my attention on the anatomy of the brain of *Carassius auratus* (L.) which is usually believed to be the ancestral form of the other two forms mentioned above.

In this paper, I have limited my observation to the principal fiber tracts of the cerebrum, including the praethalamus of C. L. HERRICK,

reserving those belonging to the other divisions of the diencephalon to a future occasion.

SHELDON's work ('12) on carp was a very helpful guide. In the present thesis, I have not only adopted the nomenclature used by this author, but also his general plan of presentation.

MATERIAL AND METHODS.

1. Material.

Specimens of *Carassius auratus* (L.) used in this study were collected exclusively in the province of Miyagi. I employed those brains which showed the same form externally, because the brain of *Carassius auratus* shows considerable external variation, especially when the brains of fish collected from different localities are compared. The fish used were about 2 to 3 years old, having the entire body length of about 8-10 cm. and a brain about 0,8-1,0 cm. long, excluding the olfactory tract. The reason for using the smaller fish was that these can be conveniently handled for preserving, sectioning, etc. I may add that the fish used, though slightly smaller, were mature in every respect, and thus did not show any significant difference from the large adult, which were examined as control, even in the finer structure of the brain, and this fact is confirmed by HOLMGREN ('20) in *Osmerus eperlanus*.

2. Methods.

The fish were killed and eviscerated for removing as much blood as possible, which was especially helpful for making the Weigert-celloidin preparations.

According to the objects of examination, various different staining methods were naturally tried :

a) HEIDENHAIN's iron-hematoxylin method.

This method gave a good result for tracing the nervus terminalis and some other non-medullated fibers. Fixation was made with 10% formol.

b) DELAFIELD's eosin-hematoxylin method.

It was least satisfactory for the teleostean cerebrum, where the medullated fibers are least abundant, but gave very good results with the medulla oblongata and other portions where the fiber tracts were

well developed. Corrosive sublimate saturated in 5% acetic acid was used as the fixative.

c) Staining *in toto* with borax-carmin.

This method was found to be useful for a rough orientation of various structures, but it was not as satisfactory as the toluidin blue method, for instance, for studying nerve cells. Staining was preceded by fixation with 10% formalin.

d) Toluidin blue method.

The fresh brain was immediately fixed in 85% or 95% alcohol for 3-5 days and imbedded in paraffine. The sections were stained with 0,1% solution of toluidin blue, and then, differentiated by anilin-alcohol. The staining fades away before long and thus is not permanent.

e) Pal-Weigert method.

Both the celloidin and paraffine imbedding methods were used. The former method was usually used for medullated nerve fibers, while the latter, which was recommended by SHELDON ('12, '14), for some special purpose, gave a good result not only for the tracing of the medullated nerve fibers, but also for the non-medullated fibers as well.

f) CAJAL's method.

SHELDON has obtained good results especially in the cerebrum of the carp. I used two modifications, one of which uses alcohol and the other does not. (SPIELMEYER, '24). The latter gave better results than the former.

II. ANATOMY.

1. *Gross anatomy.*

The cerebral hemispheres of *Carassius auratus* (L.) show closer similarity to those of the carp.

The olfactory bulbs are not directly in contact with the cerebral hemispheres such as seen for instance with *Salmo fario* and *Anguilla vulgaris* (LISSNER), but are connected by the somewhat longer olfactory tracts as in carp. (Pl. XX, Fig. 1.)

Each cerebral hemisphere exhibits an ovoidal shape, but the cerebral hemispheres together present a sub-quadrangular outline when seen from the dorsal side.

The cerebral hemispheres, the olfactory tracts and the olfactory

bulbs are covered by a common membrane, the so-called pallium or
tela. This thin membranous pallium, containing no nervous elements,
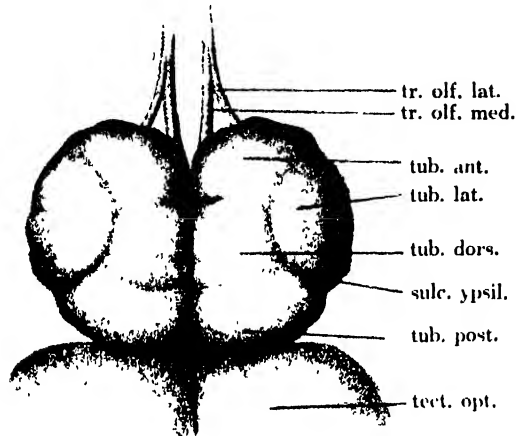
Text-fig. 1. Dorsal aspect of the cerebrum. The tela is removed
from the dorsal surface, showing clearly the tubercula.

forms the membranous roof of the cerebral hemispheres dorsally and
laterally. Extending caudally to the optic lobes and enclosing the
posterior portion, pallium makes a reflected membrane, termed the
velum transversum, at the region where the cerebral hemispheres
come in contact with the thalamus. Here, the reflected membrane
is somewhat swollen and at the same time elongated rostrally, forming
a membranous sac over the pallium proper, which sac is termed the
saccus dorsalis (Text-figs. 26, 27, 28, 29.) and originates from the
slightly caudal portion of the velum transversum in the thalamus.

    After removal of the pallium, the paired lobes over which the
various fissures run become clearly visible. These fissures, which
divide the cerebral hemispheres superficially into many small divisions,
are not so distinct as in carp. These divisions just stated are not
strictly correspondent to the divisions made by various investigators,
who attempted to divide according to the arrangement of fibers and
cells.

There is a fissure, called the sulcus ypsiliformis, which starts from the somewhat postero-lateral border of the central portion of the cerebral hemispheres, and is branched on the dorsal surface in a Y-shape.   One branch of the Y-shape proceeds towards the anterior part (the anterior limb of SHELDON) and the other proceeds towards the median line (the posterior limb of SHELDON).   Another fissure arises from the anterior portion of the median line of the cerebral hemisphere and stretches backwards, then falls in with the anterior
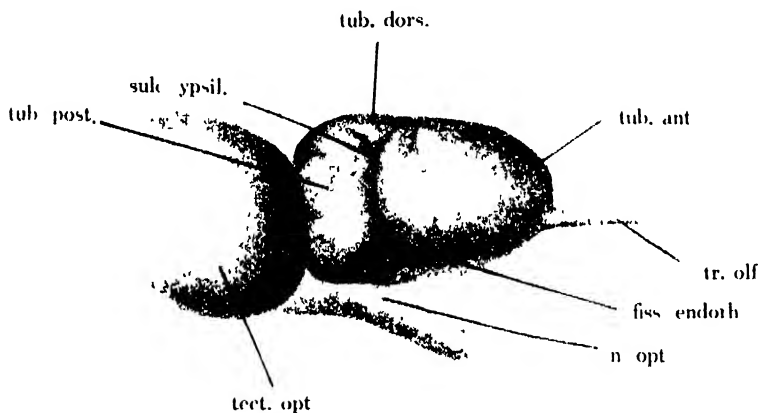
Text-fig. 2. Lateral aspect of the cerebrum. The tela is removed as in text-fig. 1.

limb of the sulcus ypsiliformis of SHELDON ('12). The fissure which arises from the median line, is correspondent to the frontal fissure of C. L. HERRICK ('91).

Moreover, at the more anterior portion, the ascending fissure of the anterior limb of the sulcus ypsiliformis bends laterally. The anterior portion surrounded by both this fissure and the frontal fissure of C. L. HERRICK is called the tuberculum anterior (Text-figs. 1, 2.).

The tuberculum dorsalis is surrounded by the frontal fissure and the antero-medial fissure (anterior limb) of the sulcus ypsiliformis. The tuberculum lateralis which occupies the lateral portion, is bordered by two branches of the sulcus ypsiliformis. The remaining tubercle other than those three tubercula just mentioned, is named the tuber-

culum posterior and is situated posterior to the sulcus ypsiliformis.

Although these external fissures are not strictly in correspondence with the anatomical divisions of the cerebral hemispheres, yet these may be utilized with advantage, roughly to divide nuclei into groups. For instance, the tuberculum anterior contains a part of the corpus precommisurale, the tuberculum dorsalis contains the nucleus olfactorius lateralis, the palaeostriatum and a part of the corpus precommissurale and the tuberculum lateralis contains the nucleus olfactorius lateralis.

On the ventral surface, the fissura endorhinalis appears as a groove,

tr. olf. med.
tr. olf lat.
tela
fiss. endorh.
sulc. ypsil.
tub. post.
chias opt.
lob. med. hyp
tect. opt.
lob. inf.
corp mamm
(GOLDSTEIN)
med. oblong.
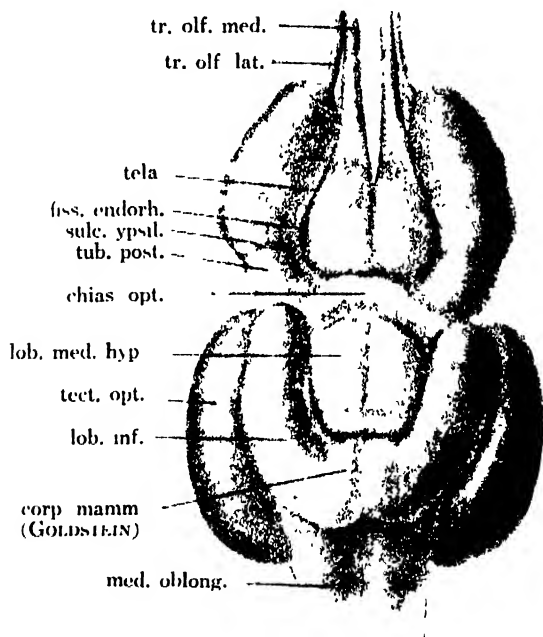
Text-fig. 3. Ventral aspect of the cerebrum The hypophysis is removed from the ventral surface.

which runs from the rostral pole and proceeds down to the point where the optic nerve decussates at the diencephalon. The groove meets laterally with the sulcus ypsiliformis, forming an open V-shape.

(Text-fig. 3.)   This is the deepest groove in the cerebral hemispheres, and is identical with the fovea limbica of EDINGER and of GOLDSTEIN, the sinus rhinalis of C. L. HERRICK and of KAPPERS, and is also assumed by these authors as homologous to the sinus rhinalis of higher vertebrates.

On the ventral surface, surrounded by the fissura endorhinalis laterally and continuous to the praethalamus of C. L. HERRICK, there are two clearly defined lobes, the anterior ends of which meet with the olfactory tracts.   These lobes are a part of the basal lobes including internally the corpus precommissurale of the secondary olfactory centers.

There is another fissure, which I believe is homologous to the sulcus limitans telencephali of SHELDON ('12) in the carp, extending longitudinally over the medio-lateral walls of the tuberculum anterior, tuberculum dorsalis, and tuberculum posterius. This fissure is very shallow, but constant in all individual fishes. This sulcus separates externally the corpus precommissurale and the nucleus olfactorius dorsalis in the basal lobe (Text-figs. 11, 16, 17, 19), and it may be homologous to the sulcus limitans hippocampi of JOHNSTON ('11).

The ventricle of the cerebrum remains as a shallow space between the pallium and the basal lobes. This space communicates anteriorly with the space which exists in the olfactory bulbs and in the olfactory tracts, and posteriorly with the third ventricle.

As to the ventral dividing line between the cerebrum and diencephalon of the teleostean brain, it has not yet been definitely agreed upon by many investigators. In this paper, the dividing line just mentioned was placed on the caudal end of the cerebrum, which is dorsally bounded by the velum transversum and encloses ventrally the praethalamus of C. L. HERRICK, where the optic nerves enter the diencephalon.   The praethalamus of C. L. HERRICK in teleostean brain contains the nucleus preopticus, which seems to me to be grouped into a tertiary olfactory center.   Therefore, I have included the praethalamus of C. L. HERRICK into the cerebrum, as SHELDON ('12) has done with the brain of carp.

2.   *Histological observations.*

(A)   Nuclei.

The nuclei, as a whole, show a similar arrangement to those of

the carp studied by Sheldon ('12). Accordingly, I adopted the terms which were used by him in his elaborate studies just mentioned.

(a)   Palaeostriatum. (Sheldon, '12).

The term " palaeostriatum " is synonymous with the corpus striatum used by many other investigators (Edinger, Goldstein, Kappers, etc.).

The palaeostriatum occupies the greater part in the central portion of the cerebral hemisphere, and takes a somewhat dorsal course gradually as it extends towards the caudal region (Pl. XX, XXI, Figs. 2, 3, 4. and Text-figs. 13, 14, 16, etc.).   It is surrounded mesially by the corpus precommissurale, dorsally by the nucleus olfactorius dorsalis, and laterally by the nucleus olfactorius lateralis.   The cell bodies which are located in the central portion appear to be largest not only within this nucleus but even when compared with the cells found in other nuclei so far studied, and are sparsely distributed.

The form of this area closely resembles that of carp, but the
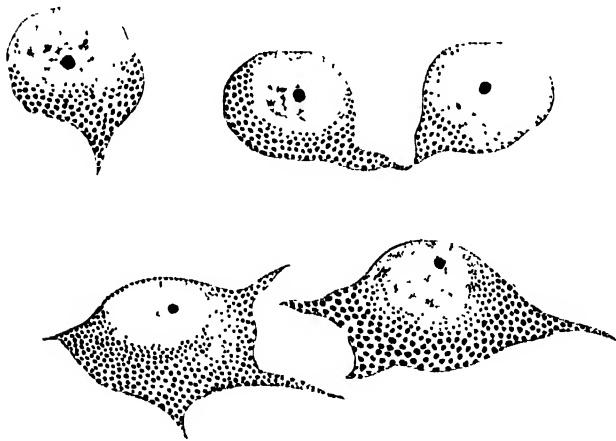
Text-fig.   4.
Cells of the palaeostriatum

arrangement of cells in the rostral portion shows much more condensation than in the carp.

(b)   Corpus precommissurale. (Sheldon, '12).

This nucleus occupies the area from the most rostral end of the cerebral hemisphere to the diencephalon, along both sides of the median cavity (ventriculus medialis), forming a well delineated column of cell groups.   Laterally the nucleus is bounded by the palaeostriatum and latero-dorsally by the nucleus olfactorius doralis.

In the cross section of the rostral part, the cell mass occupies the dorsal region of the tractus olfactorius medialis (Pl. XX, Fig. 1.). The arrangement of the cells in this nucleus is very compact and the size of cells is very small.

The relative position of this nucleus in *Carassius auratus* entirely corresponds to that of carp described by SHELDON ('12), but is not entirely identical with the epistriatum or area olfactoria posterior medialis, which was described by KAPPERS ('06) in teleosts other than carp, for the latter includes a part of the region of the nucleus olfactorius dorsalis. This nucleus appears to be homologous to the pars medialis lobi olfactorii posterioris of GOLDSTEIN ('05), while the "Vordere Nucleus" called by HALLER ('98) is a part of the corpus precommissurale.

The corpus precommissurale may be divided into four parts (four nuclei), according to the position of cell groups.

(i) The nucleus medianus (SHELDON, '12).

Among the four nuclei, the nucleus medianus occupies the most rostral end of the basal lobe in front of the anterior commissure. (Pl. XX, Figs. 1, 2, and Text-fig. 5 (A).).

(ii) The pars commissuralis (SHELDON, '12).

This pars commissuralis occupies the same position that the anterior commissure occupies, (Pl. XX, XXI Figs. 4, 5, and Text-figs. 5 (B), 19, 20, etc.).

(iii) The pars supracommissuralis (SHELDON, '12).

The pars supracommissuralis extends dorsally to the sulcus limitans telencephali and caudally to the nucleus intermedius, which is described below. (Pl. XX, XXI, Figs. 4, 5, and Text-figs. 5 (c), 19, 20, etc.).

(iv) The nucleus intermedius (SHELDON, '12).

It occupies the area from the pars supracommissuralis to the diencephalon (Pl. XXI, Fig. 6 and Text-figs. 5 (D), 24, 25).

(c) Nucleus olfactorius lateralis (SHELDON, '12).

The nucleus olfactorius lateralis has been recognized already by

many authors and has been named variously : that is, the area olfactoria
(EDINGER, HALLER, KAPPERS), the hippocampal lobe (C. L. HERRICK),
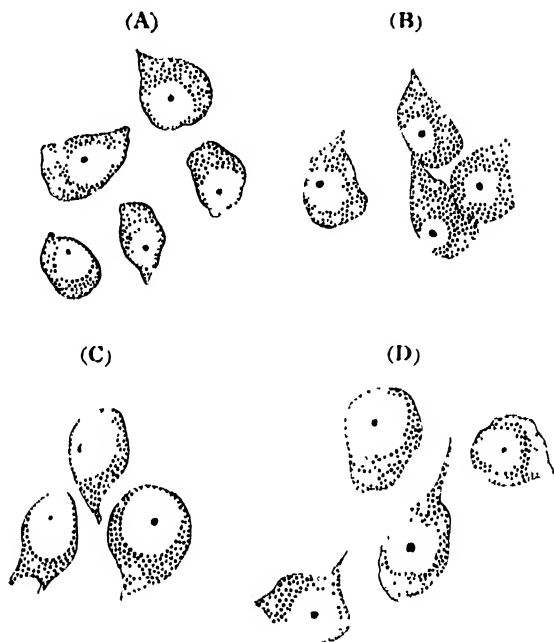
Text-fig. 5.

(A) Cells of the nucleus medianus.   (B) Cells of the pars commissuralis.
(C) Cells of the supricommissuralis.   (D) Cells of the nucleus intermedius.

the hypostriatum (CATOIS), the area olfactoria lateralis (KAPPERS and
THEUNISSEN), the area olfactoria posterior lateralis (KAPPERS) and the
pars olfactoria lateralis lobi olfactorii posterioris (GOLDSTEIN).

This nucleus is the one which occupies the largest area in the
basal lobe, bounded medially by a somewhat concave line connecting
the fissura endorhinalis to the sulcus ypsiliformis, dorso-laterally by
the palaeostriatum and latero-ventrally by the tractus olfactorius
lateralis (Pl. XX, Figs. 2, 3). Thus, this nucleus almost entirely occupies
the wide area from the rostral end of the basal lobe to the extreme
caudal pole.

'   At the latero-ventral portion of the nucleus olfactorius lateralis

proper, is found a less differentiated group of cells, which is called the nucleus pyriformis by SHELDON ('12). In *Carassius auratus*, however, I was unable to distinguish this special group of cells (the nucleus pyriformis) from the nucleus olfactorius lateralis proper, by the form and arrangement of the cells, though this nucleus is well illustrated in carp by SHELDON ('12).

Text-fig. 6.

Cells of the nucleus olfactorius lateralis

(d) Nucleus olfactorius dorsalis or primordium hippocampi (SHELDON '12).

The nucleus olfactorius dorsalis lies latero-dorsal of the sulcus limitans telencephali and dorsal of the palaeostriatum (Pl. XX, Figs. 2, 3, 4).

This area is not very clear in *Carassius auratus* for distinguishing it from the nucleus olfactorius lateralis, but it may be distinguished by the smaller size of the cell bodies from the palaeostriatum.

Text-fig. 7.

Cells of the nucleus olfactorius doralis.

(e) Nucleus teniae (KAPPERS, EDINGER, GOLDSTEIN, SHELDON).

This nucleus teniae is named the nucleus occipito-basalis by C. L. HERRICK. At the level of the anterior commissure, there appears the nucleus teniae, consisting of cells, which form a rather compact arrangement in the same manner as in the carp (SHELDON, '12). The cell mass of this nucleus begins with a small mass of cells above the fissura endorhinalis and extends caudad occupying the same relative position with this fissure just mentioned and with the ventral portion of the nucleus olfactorius lateralis. It meets the nucleus intermedius in extreme caudad (Pl. XX, XXI, Figs. 4, 5, and Text-fig. 22).

Text-fig. 8.

Cells of the nucleus teniae.

(f) Nucleus commissuralis lateralis (SHELDON. '12).
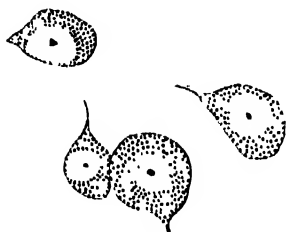
Text-fig. 9.
Cells of the nucleus commissuralis
lateralis.

The nucleus commissuralis lateralis lies slightly medio-ventrad of the fissura endorhinalis, occupying the ventral portion of each basal lobe (Pl. XX, XXI, Figs. 1, 5).

SHELDON ('12) states that " No references to it in the literature have been noted ; it has, therefore, been termed the nucleus commissuralis lateralis owing to its location, at the level of the anterior commissure."

In *Carassius auratus* this nucleus can be clearly seen in nearly the same position as was illustrated and described by SHELDON ('12).

(g) Nucleus preopticus (C. L. HERRICK, JOHNSTON, SHELDON).

The nucleus preopticus lies between the anterior commissure and the chiasma opticus, forming a part of the praethalamus of C. L. HERRICK, the portion identical to which was described with ' das zentrale Höhlengrau ' of GOLDSTEIN ('05).

Surrounding the recessus preopticus, it is in contact dorsally with the nucleus intermedius on either side (Pl. XXI, Fig. 6 and Text-figs. 22, 23 etc).

We can divide this nucleus into two parts according to the size of the cells.

(i) Nucleus magnocellularis strati grisei (GOLDSTEIN, '05).

The nucleus magnocellularis strati grisei is identical with the nidulus preopticus (C. L. HERRICK '92) and the pars magnocellularis of the nucleus preopticus (SHELDON, '12).

Closely packed, the cell mass of the nucleus magnocellularis is located adjacent to the recessus preopticus. These cells are larger than those of the nucleus parvocellularis.

(ii) Nucleus parvocellularis strati grisei (GOLDSTEIN, '05).

This nucleus parovocellularis strati grisei seems to be identical with the nucleus postopticus (C. L. HERRICK) and pars parvocellularis of the nucleus preopticus (SHELDON).

The cells of the nucleus parvocellularis are somewhat diffusely

arranged and contrasted with the crowded cell mass of the nucleus magnocellularis. The position of both these nuclei in the praethalamus is identical with that in carp (SHELDON), although in *Carassius auratus* the small cells of the nucleus parvocellularis strati grisei appear to be more or less mingled with the larger cells of the nucleus magnocellularis strati grisei, thus presenting a slightly greater irregularity in the cell arrangement than SHELDON ('12, Fig. 71.) has shown in the carp.
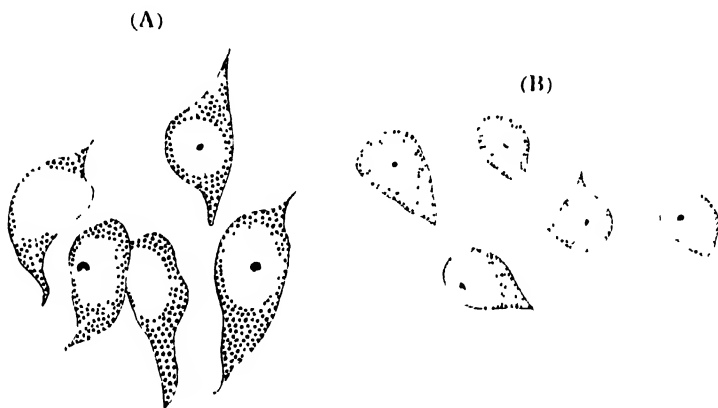
Text-fig. 10. Cells of the nucleus preopticus.
(A) Nucleus magnocellularis strati grisei.
(B) Nucleus parvocellularis strati grisei.

(h) Nucleus entopeduncularis (GOLDSTEIN, '05).

GOLDSTEIN made first observation on this nucleus in teleosts, and states that "der (nucleus entopeduncularis in teleosts) wohl mit dem von EDINGER bei Reptilien an gleicher Stelle beschriebenen Nucleus entopeduncularis identisch ist".

SHELDON also noted this nucleus in carp as identical with that of GOLDSTEIN. In *Carassius auratus* this nucleus is found at the same relative position described by these two writers. This nucleus is composed of rather smaller cells, surrounding each of the basal forebrain bundles (fasciculus lateralis hemisphaerii) at the level of the nucleus preopticus (Pl. XXI, Fig. 6).

Text-fig. 11.
Cells of the nucleus entopeduncularis.

(B) Fiber tracts.

(1) Tractus olfactorius lateralis (SHELDON, '12).

This tractus olfactorius lateralis is identical with the radix lateralis (C. L. HERRICK), laterale Riechstrahlung (EDINGER, GOLDSTEIN), tractus bulbo-corticalis (EDINGER, KAPPERS), and faisceau externe (CATOIS).

As is usually the case with some teleosts, the tractus olfactorius lateralis can be clearly separated from the tractus olfactorius medialis in *Carassius auratus*.

The tractus olfactorius lateralis, which orginates largely from the mitral cells placed laterally in the olfactory bulbs, is found on the latero-ventral part of the base of the basal lobe (Text-figs. 12, 13), and consists entirely of fibers of centripetal nature (SHELDON '12).

tractus. olf. asc.+tr. olf. med., pars lat.

tela.

n. term.

tr. olf. med.

tr. olf. lat.
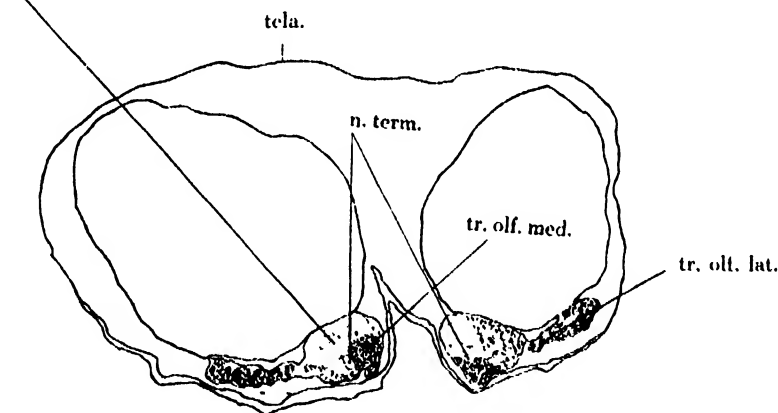
Text-fig. 12. Transection through the most rostral end of the cerebral hemispheres. The nervus terminalis appears like a hole. WEIGERT-celloidin method. ×36.

This tract consists of three components of the fibers; that is, the pars lateralis, pars intermedia, and pars medialis. As these advance caudally, it becomes difficult to distinguish these three parts from one another, and all ultimately enter the basal lobe (Text-fig. 13).

From the figure given by SHELDON in the carp (SHELDON, '12. Fig. 21), three parts of the tractus olfactorius lateralis can be distinguished from one another at the rostral portion of the basal

tr. olf. asc.
+tr. olf. med., pars lat.   tela   palaeostr.

f. endorh

tr. olf. med., pars med
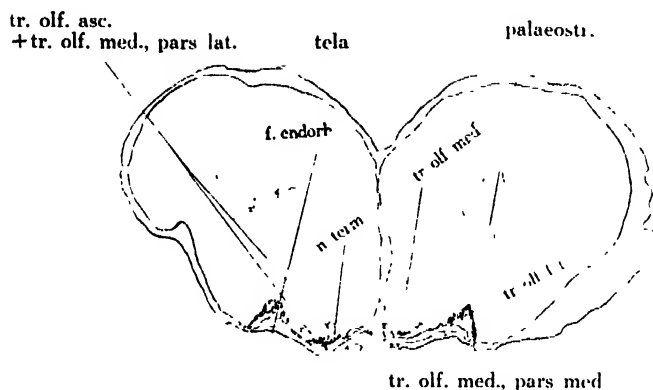
Text-fig. 13. Transection through the rostral portion of the cerebral hemispheres, at which the tractus olfactorius enters the basal lobes The fibers from the palaeostriatum are shown in this section. WEIGERT-celloidin method. ×21.

lobe, much more easily than in *Carassius auratus*. After entering the basal lobe, this tract can be clearly found above the fissura endorhinalis (Text-figs. 12, 13). A part of the tractus olfactorius lateralis terminates in the nucleus medianus of the corpus precommissurale as is seen in horizontal sections, while the remainder enters into the nucleus olfactorius lateralis, where the connection is established with the neurons of the second order.

tela.   palaeostr   lim. tel.
tr. olf lat , pars med (?)
sulc ypsil
tr. olf. med.,
pars lat
tr. strio-thal
nucl. medianus

f. endorh.   tr. olf. lat
n. term.   tr. olf. med., pars med.
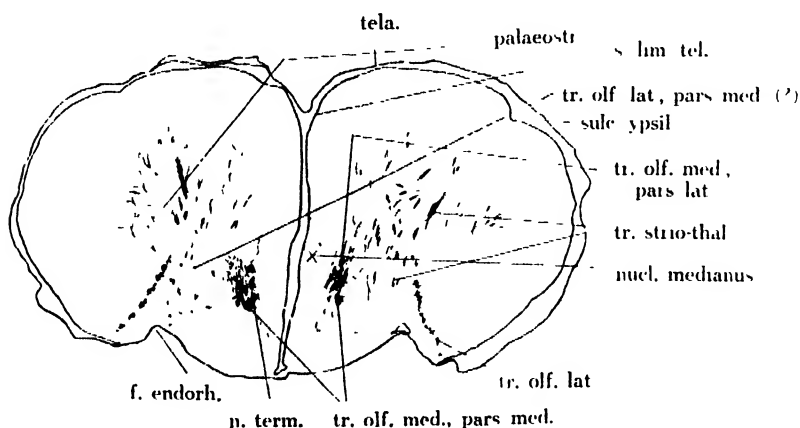
Text-fig. 14. Transection through the portion slightly rostral to the anterior commissure. WEIGERT-celloidin method. ×21.

In the secondary olfactory area are included, as in carp, the nucleus pyriformis of the nucleus olfactorius lateralis as well as the nucleus teniae.

The statement made by SHELDON ('12) that " the fibers of all three portions of the tractus olfactorius lateralis... ......gradually spread out above the fissura endorhinalis, without decussating, in the lateral olfactory area of the basal lobes ", requires in my mind further investigation of experimental nature, because the most medial portion (pars medialis?) of the tractus olfactorius lateralis (Text-figs. 14, 15, 16, 17, 19) appears to me to decussate at the anterior commissure in *Carassius auratus* and this fact coincides with the case in some teleosts, including the carp, described by GOLDSTEIN ('05. Text-fig. 5).

(A)

- n. term.

tr olf. lat , pars med. (?).

tr. olf. lat , pars lat.

- corp. precomm.

— nucl, olf. lat.

(B)

n. term.
tr. olf. med., pars med.
tr. olf. ascendens.
tr. olf. med., pars lat.

- corp precomm.

- nucl. olf. lat.
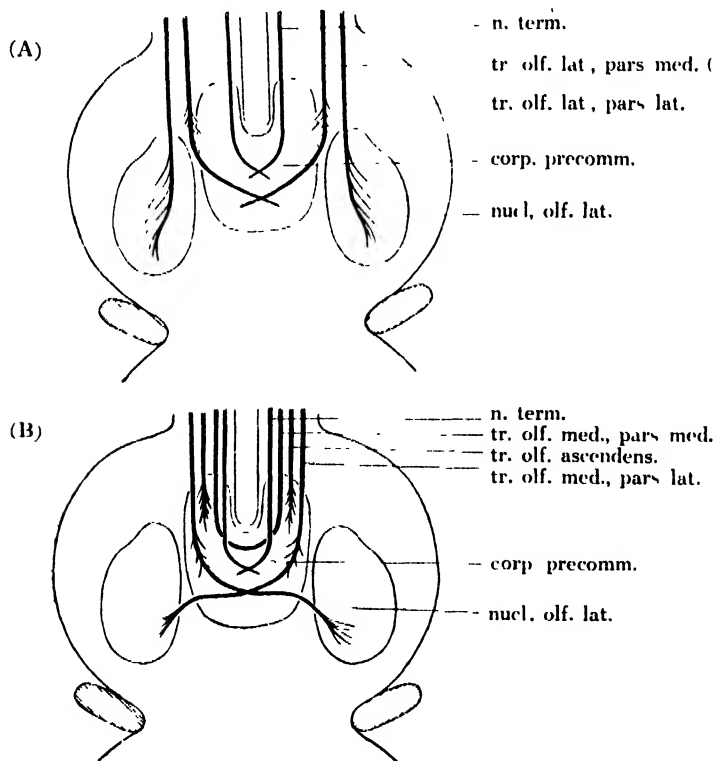
Text-fig. 15   Horizontal scheme of the fore-brain tracts.
(A) Tractus olfactorius lateralis.   (B) Tractus olfactorius medialis.

Quoting from KAPPERS ('06) we read that " In an adult *Thynus* and some other *Lophius*, however, I again found the uncrossed tract so that we must assume that both occur and that probably the uncrossed course of the lateral fibers is the more general one, as it has been described by `BELLONCI, EDINGER and C. L. HERRICK. It would be interesting to find out the conditions which determined this different behavior." Even if we assume with KAPPERS that this tract shows the decussation in some cases and non-decussation in others, it seems highly improbable that the carp and *Carassius auratus*, both of which show closer general arrangement of the fiber tracts, totally differ in this tractus olfactorius lateralis, which shows non-decussation in carp and decussation in *Carassius auratus*.

(2) Tractus olfactorius medialis (SHELDON, '12).

This tractus olfactorius medialis is identical with the radix mesalis (C. L. HERRICK), tractus bulbo-epistriaticus (EDINGER), mediale Riech-strahlung (EDINGER, GOLDSTEIN), faisceau interne (CATOIS).

Many authors considered all of these tracts as consisting of centri-petal fibers, but CATOIS ('02) in *Conger vulgaris* and SHELDON ('12) in carp state that this tract contains both centrifugal and centripetal bundles.

CATOIS describes "......sa partie interne renferme de nombreuses fibres à direction centrifuge," but SHELDON observed this centifugal bundle in the central portion of the tractus olfactorius medialis. This tract can be divided into the following groups of fibers : —

    (a) Centrifugal fibers........Tractus olfactorius ascendens
                                    (SHELDON, '12).
    (b) Centripetal fibers.. ....Tractus olfactorius medialis pars
                                      lateralis. (SHELDON, '12).
    (c) Centripetal fibers........Tractus olfactorius medialis pars
                                      medialis. (SHELDON, '12).

(a) Tractus olfactorius ascendens (SHELDON, '12).

The tractus olfactorius ascendens, which is considered by SHELDON as composed of centrifugal fibers, constitutes anatomically a part of the so-called radix olfactoria medialis propria of KAPPERS ('06), which is considered by him as composed of centripetal fibers. Although in the carp the tractus olfactorius ascendens is distinguished from the tractus olfactorius medialis, even at the rostral end of the basal lobe,

such distinction is impossible in *Carassius auratus*, and both appear
to be mingled in this region. (Text-fig. 12, 13).

The tractus olfactorius ascendens accompanies the tractus olfactorius
medialis up to about the portion slightly rostral to the anterior com-
missure (Text-fig. 14), and then separates from the tractus olfactorius
medialis proper.   This tract originates from the nucleus medianus of
the corpus precommissurale as in carp and is located nearer to the
median cavity (Text-figs. 16, 17).   This tract probably corresponds to
' der in der Mitte liegenden Faserbündel ' of GOLDSTEIN ('05), although
he considered it as composed of centripetal fibers.

tela s. lim. tel   vent. med.

sulc. ypsil.
nucl. medianus
tr. olf. asc.
(origin)
palaeostr.

tr. strio-thal.

tr. olf. lat.

t. endorh.   tr. olf. med., pars lat.
tr. olf. lat.,   tr. olf. med., pars med.
pars med. (?)   (com. interbulb)
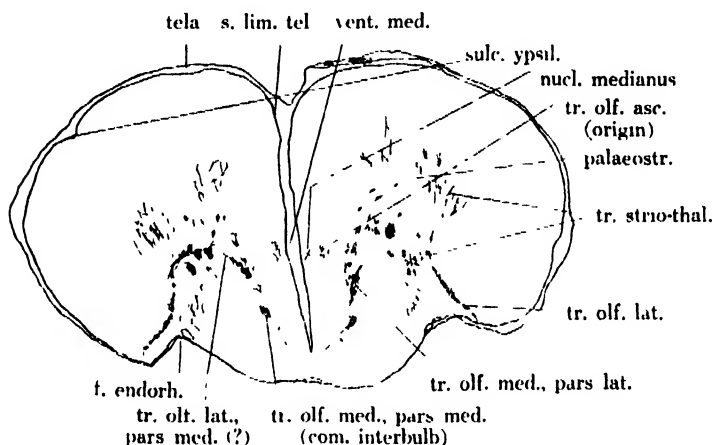
Text-fig. 16.   Transection through the portion immediately caudal
to the level of text-fig. 14. WEIGERT-celloidin method. ×24

(b) Tractus olfactorius medialis pars medialis (SHELDON, '12).

In *Carassius auratus*, the tractus olfactorius medialis pars medialis
occupies a part of the tractus olfactorius medialis forming a distinct
fiber bundle as in carp, as shown in Text-figs. 12 and 13.   After
reaching the basal lobe, it holds a medio-ventral position accompanying
the tractus olfactorius medialis proper (Text-figs. 13, 14).   This pars
medialis, which runs dorsad after separating from the tractus olfactorius
medialis, is situated just on the ventral side of the tractus olfactorius
medialis pars lateralis.   In a further caudal region, it enters the
anterior commissure, occupying a more ventral position than that of
the tractus olfactorius medialis pars lateralis.

The majority of the fibers in this tract, which forms a part of the anterior commissure and is located more ventrally than rest of the commissural fibers, are seen to connect the two olfactory bulbs (Text-figs. 11, 15, 16, 17, 18), as were shown also by both GOLDSTEIN and SHELDON in carp. This commissure is termed the commissura olfactoria interbulbaris (GOLDSTEIN, '05).

The rest of the fibers of the tractus olfactorius medialis pars medialis seems to end, without decussating, in the nucleus medianus as well as in the pars commissuralis of the corpus precommissurale.
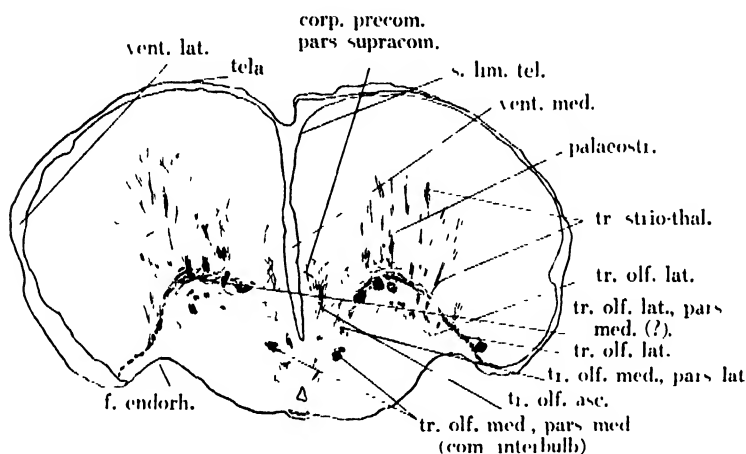
Text-fig. 17. Transection through the portion in front of the anterior commissure This section shows the tractus olfactorius medialis pars medialis as made up of the fibers of the commissura olfactoria interbulbaris. WEIGERT-celloidin method. ×24.

(c) Tractus olfactorius medialis pars laterialis (SHELDON, '12).

The tractus olfactorius medialis pars lateralis occupies the lateral portion of the tractus olfactorius medialis, but it is very difficult to distinguish, owing to less differentiation of staining reaction, from the tractus olfactorius ascendens at the rostral end of the basal lobe (Text-figs. 12, 13).

This tract becomes distinct at about the portion rostral to the anterior commissure (Text-fig. 11), and occupies the dorsal portion of the tractus olfactorius medialis pars medialis, then, gradually takes a dorso-lateral course. The greater part of these fibers, which form a

part of the anterior commissure, end into the nucleus pyriformis of the opposite side, though a part of them seems to terminate in the nucleus teniae. The smaller number of the fibers in the tractus olfactorius medialis pars lateralis end into the pars supracommissuralis of the corpus precommissurale. My own observations, in *Carassius auratus*, thus correspond essentially with the observations of SHELDON on carp, with the exception that in the former there were not found the fibers which end into the nucleus olfactorius dorsalis.

The tractus olfactorius medialis pars lateralis agrees with the 'Fasern aus dem lateralen Teil der medialen Riechstrahlung' of GOLDSTEIN ('05).
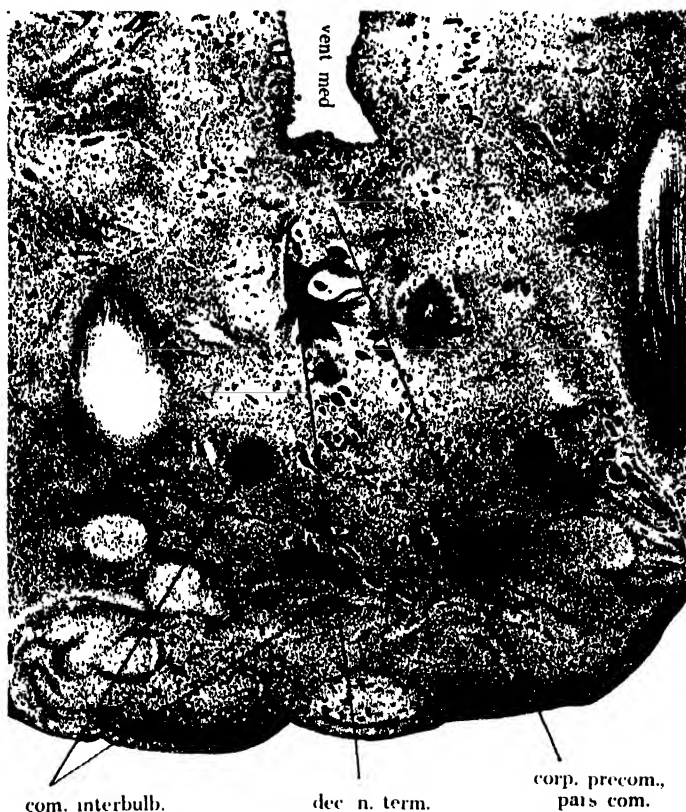
Text-fig. 18. Transection through the anterior portion of the anterior commissure for showing the decussation of the nervus terminalis. HEIDENHAIN's iron-hematoxylin method.' ×124.

(3) Nervus terminalis (LOCY, '05) or PINKUS' nerve (PINKUS, '94).

The nervus terminalis is located along and imbedded in the middle portion of the tractus olfactorius medialis. In the WEIGERT-celloidin preparations it remains an unstained clear area in the cross section, owing to non-medullation of the fibers, though the WEIGERT-paraffine preparations may barely show the fibrous character of this area (Text-figs. 12, 13, 14).

The nervus terminalis, though its origin is not yet determined in this present study, is found to accompany the tractus olfactorius medialis pars medialis, and then separates from the latter, placing itself slightly ventro-lateral to the median cavity.

Although I could not trace the entire course of this nerve, it probably passes in the most medial portion along the median cavity, as SHELDON noted in carp and HOLMGREN ('18) in *Osmerus*, because much of the course so far followed, corresponds with the descriptions of these two authors mentioned.

The decussating point of the nervus terminalis lies on the more rostral end of the anterior commissure and slightly caudad of the commissura olfactoria interbulbaris of GOLDSTEIN ('05), or, in general, it lies about the middle between the commissura olfactoria interbulbaris and the decussation of the tractus strio-thalamicus cruciatus (*vide* (i), pp. 509 *et seq.*)

(4) Commissura anterior.

The two basal lobes in *Carassius auratus* are connected by the anterior commissure which consists of the following commisural and decussating fibers. The general arrangement of the fibers in the anterior commissure is practically the same in *Carassius auratus* as in the carp, which was fully described by SHELDON.

(a) Commissura olfactoria interbulbaris (GOLDSTEIN, '05).

The commissura olfactoria interbulbaris is formed by the fibers of the tractus olfactorius medialis pars medialis, which lies medially in the tractus olfactorius medialis, in front of the decussation of the nervus terminalis. Although these commissural fibers are considered by other investigators to unite the two olfactory bulbs, in *Carassius auratus* not all fibers appear to decussate.
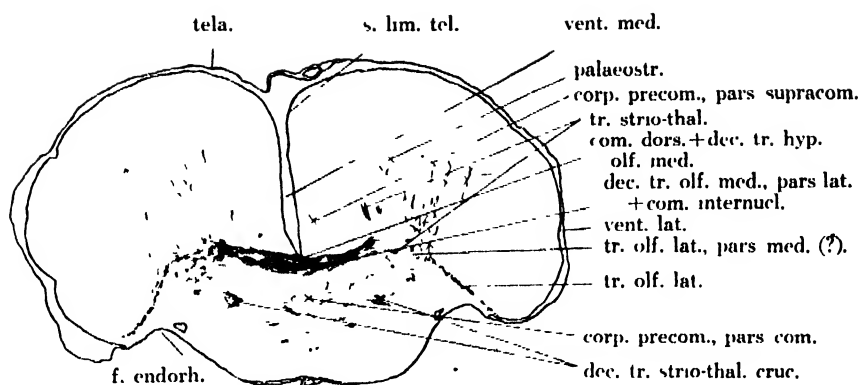
Text-fig. 19. Transection through the middle portion of the anterior com-
missure. WEIGERT-celloidin method. ×24.

(b) Commissura dorsalis.

This bundle of the commissura dorsalis is larger than the one
just described and binds the nucleus olfactorius dorsalis of the two
opposite lobes. At the uppermost layer of the anterior commissure,
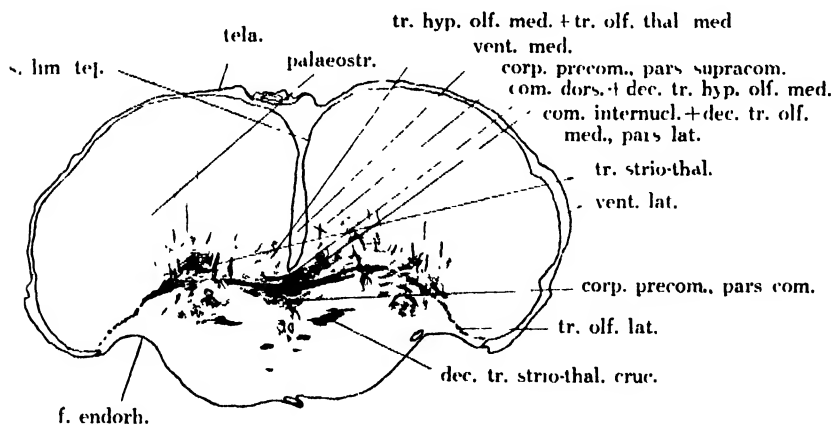
Text-fig. 20. Transection through the portion immediately caudal to the
middle portion of the anterior commissure. In this section the bundle of the
commissura olfactoria internuclearis is clearly shown. WEIGERT-celloidin method.
×24.

these commissural fibers mingle with the fibers of the tractus hypo-
thalamo-olfactorius medialis. (Text-figs. 19, 20, 21).

(c) Commissura olfactorii internuclearis (GOLDSTEIN, '05).

The commissura olfactorii internuclearis is a thick bundle which occupies the greater fraction of the anterior commissure, exhibiting bow-shaped arrangement in the cross-section (Text-fig. 20). This commissure in *Carassius auratus* is identical with the hippocampal commissure of C. L. HERRICK and the commissura olfactoria of KAPPERS. This commissure in *Carassius auratus* is undoubtedly identical with the commissura hippocampi pars posterior of SHELDON in carp in regard to its position, but he describes this as entirely non-medullated fibers, while in *Carassius auratus* it is largely composed of medullated fibers as GOLDSTEIN ('05) noted in carp and as illustrated from *Salmo* ('05, Fig. 16, Taf. XIII).
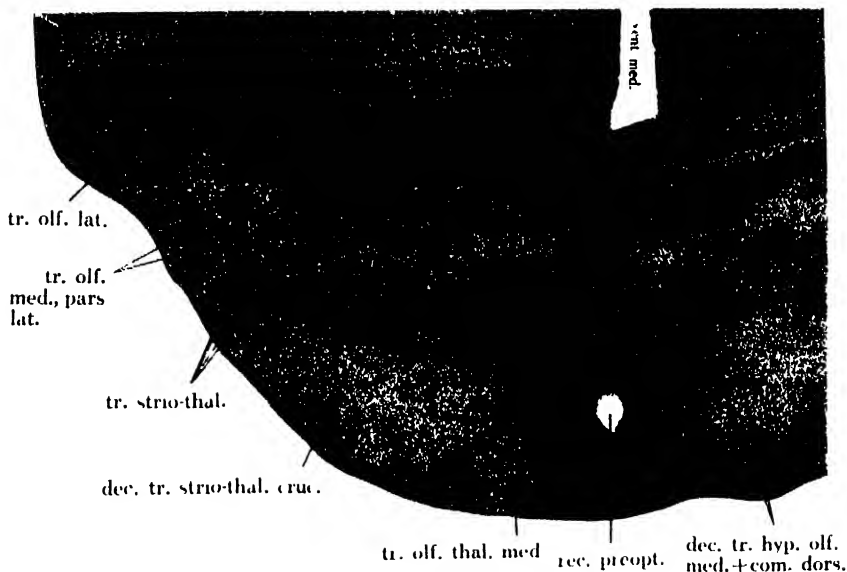
Text-fig. 21. Transection through the slightly caudal part anterior commissure. WEIGERT-paraffine method. × 7!

(d) Decussation of the nervus terminalis.

In regard to this decussation, I have already described it in connection with the nervus terminalis, and therefore the description will not be repeated.

(e) Decussation of the tractus olfactorius medialis pars lateralis.
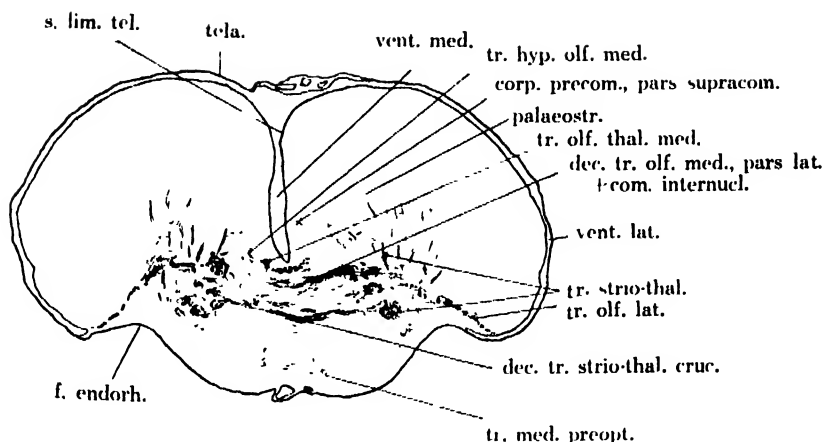
Text-fig. 22. Transection through the caudal portion of the anterior commissure. In this section the decussation of the tractus strio-thalamicus cruciatus is rather clearly marked. Weigert-celloidin method. ×24.

The fibers which are found almost in the middle portion of the anterior commissure in the horizontal section, are closely associated with the commissura olfactorii internuclearis.

(f) Decussation of the tractus strio-thalamicus cruciatus.

These decussating fibers consist of partly non-medullated and partly medullated fibers and form one of the largest bundles situated on the middle portion of the anterior commissure in the cross section. The bundle consists of those fibers which come from the mid-portion and from the more caudal portion of the palaeostriatum (Text-figs. 19, 21, 22, 23).

(g) Decussation of the tractus hypothalamo-olfactorius medialis.

The fibers are derived from the hypothalamus and enter the pars supracommissuralis of the corpus precommissurale along the median cavity, passing through the anterior commissure, ventral to the fibers of the commissura dorsalis.

(5) Connecting fibers with the diencephalon.

(a) Fasciculus medialis hemisphaerii (Sheldon, '12).

In this fasciculus I can identify in *Carassius auratus* (L.) the two tracts; tractus hypothalamo-olfactorius medialis (Sheldon, '12) and tractus olfacto thalamicus medialis (Sheldon, '12), but the four other

(A)

dec tr. hyp olf med.

com dorsalis
dec tr olf. med, pars lat.

com. olf. internucl.

dec tr strio thal cruc

dec n term

com. olf interbulb

(B)

dec n term
com olf interbulb
dec tr. olf. med, pars lat

dec. tr hyp olf med
com dorsalis

com olf. internucl
dec tr strio-thal. cruc.
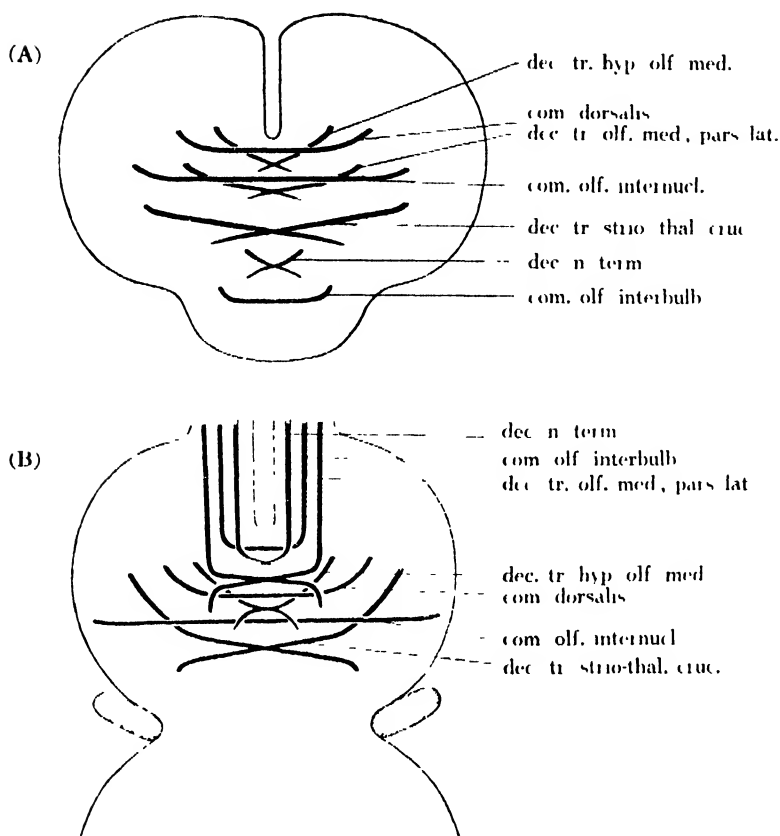
Text fig. 23.   Scheme of the anterior commissure

(A) Transverse view.   (B) Horizontal view

tracts which SHELDON was able to trace in carp were difficult to differentiate.

(i) Tractus hypothalamo-olfactorius medialis.

The tractus hypothalamo-olfactorius medialis is identical with the tractus olfacto-hypothalamicus medialis of GOLDSTEIN, tractus olfacto-lobaris medialis of KAPPERS ('06), but both the latter authors consider it as an ascending tract.

This tract largely terminates in the pars commissuralis and pars supracommissuralis of the corpus precommissurale, and the middle portion of the anterior commissure it is closely adjacent to the median

cavity (Text-figs. 20, 22). At the level of this terminal, the fibers intermingle with the fine fibers coming from the origin of the tractus olfacto-thalamicus medialis, and immediately after intermingling, these fibers of the tractus hypothamo-olfactorius medialis decussate in the anterior commissure.
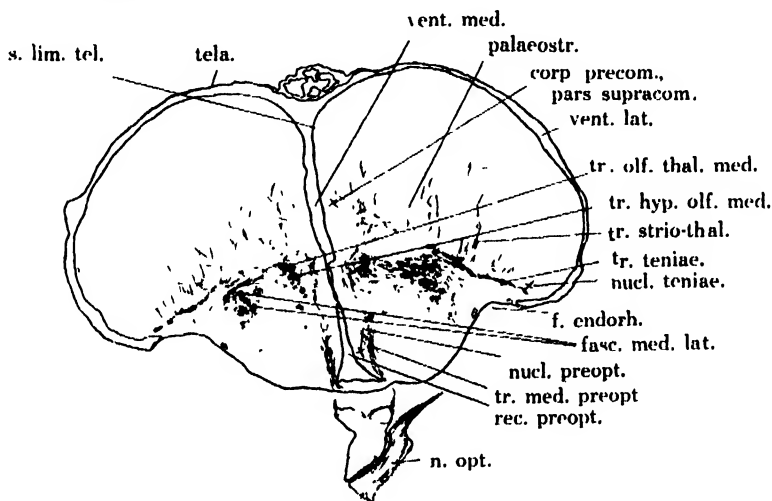
Text-fig. 24. Transection through the rostral level of the recessus pre-opticus, a little caudal to the anterior commissure. The tractus mediano preopticus is faintly shown. WEIGERT-celloidin method. ×24.

It is very difficult to draw a sharp line between this tract and the commissura dorsalis, for both tracts are intermingled intimately with each other. This tract extends caudally together with the tractus olfacto-thalamicus medialis (pars dorsalis and pars ventralis, SHELDON, '12) forming one bundle. Thus, these two tracts are called, as a whole, "basal cerebral fasciculus" by C. L. HERRICK and "fasciculus medialis hemisphaerii" by SHELDON. At the level of the chiasma opticus, this fasciculus just stated forms the "fornix tract" of C. L. HERRICK (Text-fig. 26).

As it approaches the hypothalamus, the bundle of the tractus hypothalamo-olfactorius medialis becomes finer, and then separates from the tractus olfacto-thalamicus medialis. Differentiation of the tractus hypothalamo-olfactorius medialis from the tractus olfacto-thalamicus medialis is not easy in *Carassius auratus* (L.), as is the case

in carp, since these bundles are imbedded among the fibers belonging to various undetermined tracts. These fibers originate largely from the nucleus ·posterior tuberis at the neighborhood of the nucleus rotundus (Text-figs. 31, 32).

It still remains unsettled whether this tract terminates in, as KAPPERS and GOLDSTEIN maintain, or arises from, as SHELDON reports, the nucleus posterior tuberis.
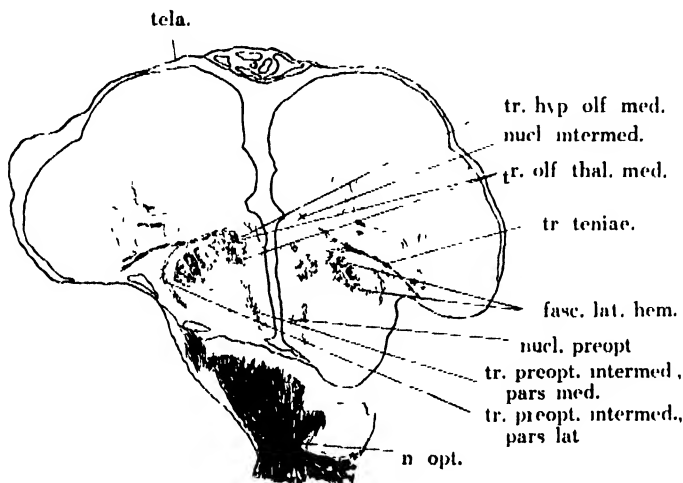
Text-fig. 25. Transection through the praethalamus of C. L. HERRICK. The fine fibers of the tractus olfacto-hypothalamicus lateralis are presented in this figure   WEIGERT-celloidin method.   × 24.

(ii) Tractus olfacto-thalamicus medialis.

These fibers originate largely from the pars commissuralis of the corpus precommissurale and appear ventrad of the decussation of the tractus olfactorius medialis pars lateralis (Text-fig. 20).

Although at the beginning of its course the tractus is placed on the ventral side of the tractus hypothalamo-olfactorius medialis, soon it accompanies the latter (Text-fig. 24). The tractus olfacto-thalamicus medialis runs along the median cavity, forming a part of the "basal cerebral bundle" of C. L. HERRICK, afterwards it enters the hypothalamus (a part of the 'fornix tract' of C. L. HERRICK.) (*vide* p. 506.)

At the level where the commissura horizontalis (FRITSCH) appears,

the tract becomes diffused, giving off its fibers into the nucleus rotundus, nucleus posterior tuberis and nucleus subrotundus (Text-fig. 31).   The terminal of this tract is located a little more laterally than that of the tractus hypothalamo-olfactorius medialis.

SHELDON ('12) states in his paper on carp that "A short distance caudal to the anterior commissure, the medial forebrain bundle has increased largely in size (figs. 68, 69), due to the presence of a large number of short fibers, . . . Another factor in the increase in size of the median bundle consists in the addition to it of a few medullated fibers arising from the dorso-lateral part of the nucleus magnocellularis, forming the tractus preoptico-tuberis.   . ".

In *Carassius auratus* (L.), however, at the level mentioned by SHELDON, there is not any remarkable difference in size, although the tractus preoptico-tuberis and tractus habenulo-diencephalicus (GOLDSTEIN, '05) associate with the fasciculus medialis hemisphaerii, as was pointed
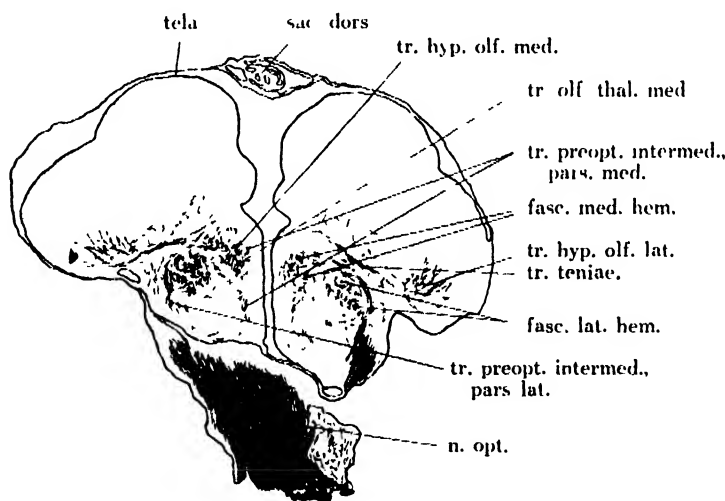
Text-fig. 26. Transection through the portion slightly caudal to the level shown in text-fig. 25. This section shows the tractus preoptico-intermedius pars medialis and pars lateralis. WEIGERT-celloidin method. ×24.

out by SHELDON. The reason why in *Carassius auratus* (L.), this increase in size does not appear, seems due to the giving off of some fibers, in the dorsal part of the diencephalon in its course.

(b) Fasciculus lateralis hemisphaerii (SHELDON, '12).

The fasciculus lateralis hemisphaerii was variously named by previous workers, as for instance, basale Vorderhirnbündel by EDINGER and by GOLDSTEIN, and faisceau basal by CATOIS, but strictly speaking, these are not entirely identical with the fasciculus lateralis hemisphaeii. This fasciculus lateralis hemisphaerii consists of the two bundles: the tractus strio-thalamicus and tractus olfacto-hypothalamicus lateralis.

(i) Tractus strio-thalamicus (GOLDSTEIN, KAPPERS, JOHNSTON, SHELDON).

This great fiber bundle of the fasciculus lateralis hemisphaerii is derived from almost all parts of the palaeostriatum, consisting both of decussating and non-decussating fibers as in other teleosts. This tractus is composed of the two smaller tracts: tractus strio-thalamicus cruciatus and tractus strio-thalamicus incruciatus. So far as the position of both these tracts are concerned, it is the same as in the case
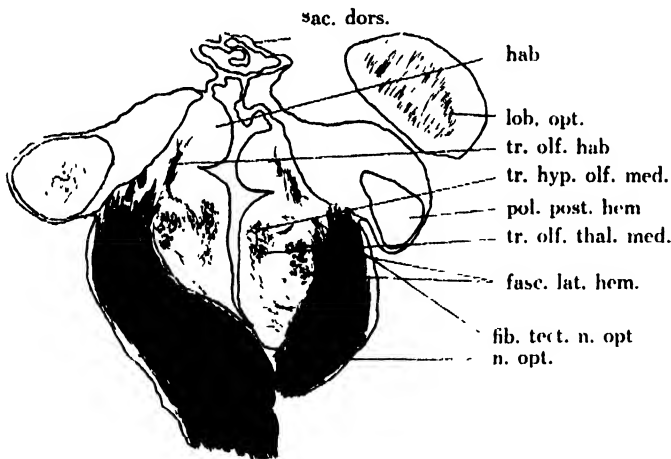
Text-fig. 27. Transection through the level of the habenula. The cerebral hemispheres are just coming to the end. WEIGERT-celloidin method. fib. tect. n opt.=fibrae tectales nervi optici. ×24.

of carp. In the anterior commissure, the tractus strio-thalamicus cruciatus is seen as a massive bundle occupying the caudo-dorsal position to the commissura olfactoria interbulbaris (Text-figs. 20, 22).

As the median cavity grows deeper and deeper towards ventral, both the decussating and some of non-decussating fibers run caudad, forming a few longitudinal bundles, while some non-decussating fibers

are seen constantly to enter the bundles of the tractus strio-thalamicus which comes from the palaeostriatum.

The bundles of the tractus strio-thalamicus lie slightly latero-ventrad to the bundles of the tractus hypothalamo-olfactorius medialis and tractus olfacto-thalamicus medialis, namely dorso-medial to the fissura endorhinalis (Text-fig. 24). In *Carassius auratus* (L.) usually, numerous non-medullated fibers are found among the medullated fibers of the tractus strio-thalamicus, as SHELDON already noted in carp.

CAJAL's preparations show clearly that the tractus strio-thalamicus cruciatus is situated dorsal to the tractus strio-thalamicus incruciatus,
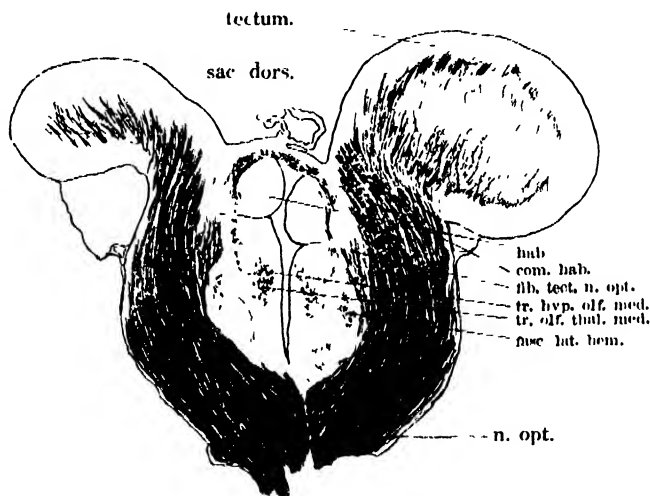
Text-fig. 28. Transection through the portion, where the optic lobes begin to appear. This section shows the commissura habenularum. WEIGERT-celloidin method. ×2⅓.

but as it proceeds in the more ventro-lateral direction, these tracts appear closely located to the optic nerve (Text-figs. 27, 28). As is shown in text-fig. 29, these bundles lie between the commmissura HERRICKI and the optic nerve, beyond which the medullated fibers of these bundles seem to diminish rapidly, thus, giving an obscure appearance to the tractus strio-thalamicus in the WEIGERT-celloidin preparations. Further caudad (Text-fig. 30), the tractus strio-thalamicus is divided into two parts. According to SHELDON, in carp this tractus

mentioned above, separates itself distinctly into two tracts: the tractus strio-thalamicus cruciatus and tractus strio-thalamicus incruciatus, and the latter is accompanied by the tractus olfacto-hypothalamicus lateralis, but is not clear in *Carassius auratus* (L.). Both branches of the tractus strio-thalamicus mentioned above end into the various nuclei which are situated near the commissura horizontalis of FRITSCH, in the ventral portion of the diencephalon composed of the nucleus rotundus, nucleus anterior tuberis, and nucleus lateralis tuberis, etc. Although the above statements on carp made by SHELDON essentially agree with mine on *Carassius auratus* (L.), he further pointed out that some fibers of the dorsal part of the tractus strio-thalamicus terminate also in the nucleus posterior thalami, nucleus cerebellaris hypothalami, and nucleus diffusus lobi lateralis, which, however, were not observed by the present writer.

(ii) Tractus olfacto-hypothalamicus lateralis et tractus hypothalamo-olfactorius lateralis (SHELDON, '12).
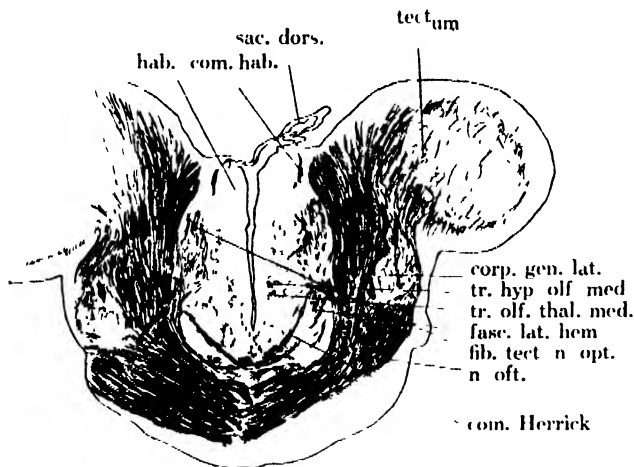
Text-fig. 29. Transection through the level of the corpus geniculatum laterale. This section shows the fasciculus lateralis hemisphaerii, approaching the optic nerve. WEIGERT-celloidin method. corp. gen. lat. = corpus geniculatum laterale. ×24.

In *Carassius auratus* (L.) this tract is quite non-medullated and the usual WEIGERT's stain can not bring out this structure clearly, and thus CAJAL's method was applied.

At the level where the fasciculus lateralis hemisphaerii in the praethalamus of C. L. HERRICK is formed by the fibers derived from the palaeostriatum, the fibers of the tractus olfacto-hypothalamicus lateralis begin to appear above the tractus teniae and the tractus preoptico-intermedius pars lateralis, just as the fibers of the tractus strio-thalamicus incruciatus enter the fasciculus lateralis hemisphaerii (Text-fig. 26).

The nucleus from which this tract originates, seems to be located in a more caudal part of the nucleus olfactorius lateralis. But I was unable to trace these fibers into the fasciculus lateralis hemisphaerii,
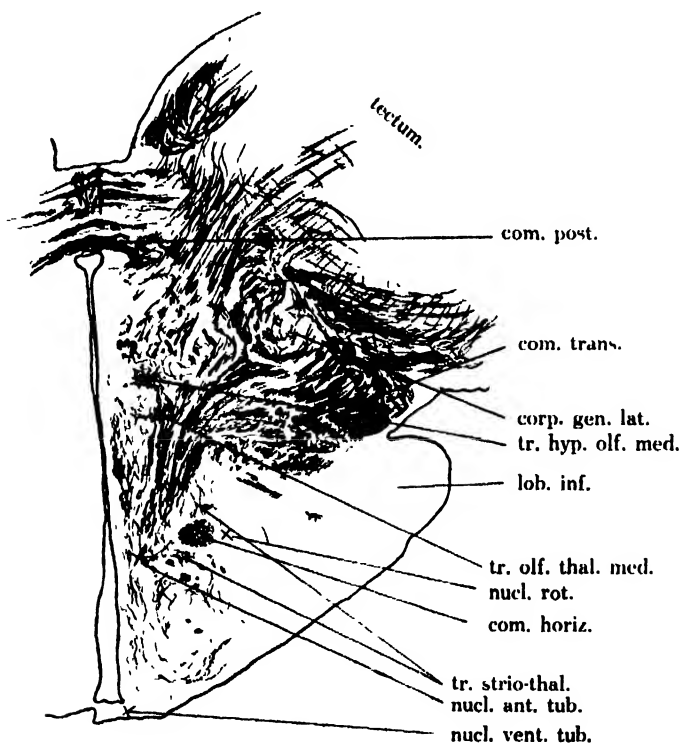
Text-fig. 30. Transection through the level of the commissura posterior. The fibers of the tractus strio-thalamicus end around the commissura horizontalis of FRITSCH. WEIGERT-celloidin method. com. post.=commissura posterior. com. trans.=commissura transversa. lob. inf.=lobus inferior. nucl. rot.=nucleus rotundus. nucl. ant. tub.=nucleus anterior tuberis. nucl. vent. tub.=nucleus ventralis tuberis. ×36.

as has been done by many previous authors.

At any rate, the tractus olfacto-hypothalamicus lateralis of *Carassius auratus* (L.) is not a very distinct one, as KAPPERS ('06) claims it in other fishes to be " a compact large tract of unmedullated fibers ", since the fibers which arise from the caudo-ventral portion of the nucleus olfactorius lateralis are not numerous and furthermore these immediately mingle with the fibers of the tractus teniae.

(c) Tractus olfacto-habenularis or tractus teniae (SHELDON, '12).

The tractus teniae in *Carassius auratus* (L.) is evidently identical with the tractus olfacto-habenularis taeniae of EDINGER, KAPPERS etc., faisceau cortico-habenulaire of CATOIS, and taeniae thalami of GOLDSTEIN.

Since I limited my observation to the region from the basal lobe to the ganglia habenularum, thus the connections from the habenular ganglia to the thalamus were not studied.

This tract appears to arise from almost all parts of the nucleus teniae, exhibiting a continuous group of fibers of the tractus olfactorius lateralis when seen in the horizontal sections.   This tract is removed gradually medio-dorsad throughout the upper part of the praethalamus of C. L. HERRICK, and then, forms the commissura habenularis (GOLD-STEIN) at the level of the portion where the optic lobes just appear (Text-fig. 28).

(d) Tractus mediano-preopticus (SHELDON, '12).

At the slightly ventral portion of the anterior commissure, the fine fibers arise both from the nucleus and the ventral part of the pars commissuralis of the corpus precommissurale, as is shown in carp. These fibers consist of both medullated and non-medullated fibers and thus can be demonstrated advantageously by WEIGERT-paraffine preparations.

At first, these fibers run straight ventralwards and appear on both sides of the recessus preopticus, then descend more ventrally and enter the network formed by the processes arising from the nucleus preopticus (Text-fig. 24), but continue their course clearly to the base of the nucleus preopticus.

(e) Tractus preoptico-intermedius (SHELDON, '12).

The positions of the parts of tractus preoptico-intermedius are indicated in text-fig. 26.   The fibers are largely non-medullated and bind the nucleus preopticus to the nucleus intermedius of the corpus
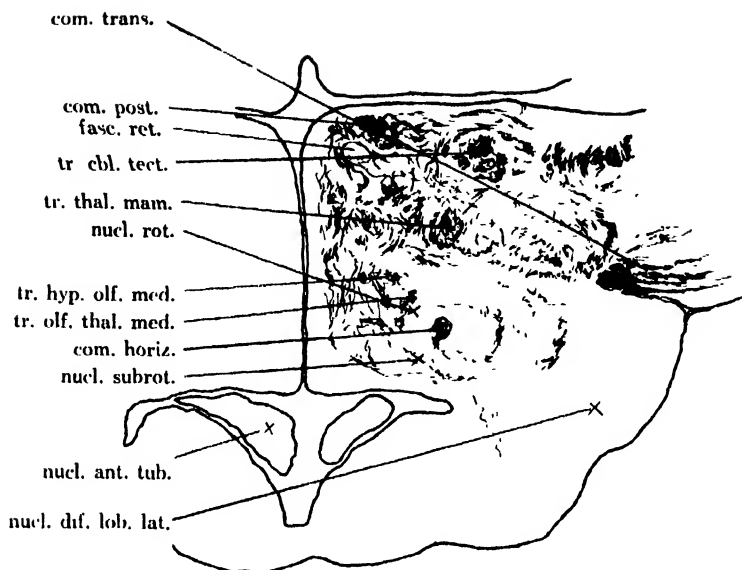
Text-fig. 31. Transection more caudal to the level shown in text-fig. 30. This section shows the origin of the tractus hypothalamo-olfactorius medialis and the terminal of the tractus olfacto-thalamicus medialis. WEIGERT-celloidin method. fasc. ret.=fasciculus retroflexus. tr. cbl. tect.=tractus cerebellotectalis. tr. thal. mam.=tractus thalamomammillaris. nucl. subrot.=nucleus subrotundus. nucl. dif. lob. lat.=nucleus diffusus lobi lateralis. ×36.
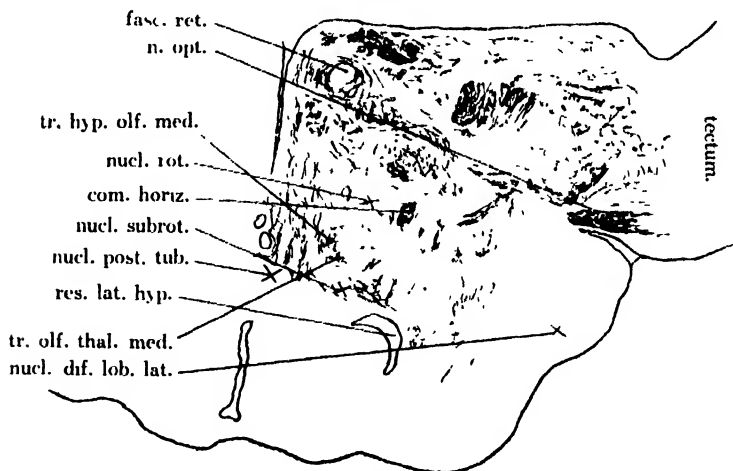
Text-fig. 32. Transection slightly caudal to the level shown in text-fig. 31. Both tracts of the tractus hypothalamo-olfactorius medialis and tractus olfacto-thalamicus medialis likewise appear, as shown in text-fig. 31. WEIGERT-celloidin method. nucl. post. tub.=nucleus posterior tuberis. res. lat. hyp.=recessus lateralis hypothalami. ×36.

precommissurale. Accordingly, it was necessary to study these fiber
tracts with the aid of CAJAL's method.

In *Carassius auratus* (L.), this tractus preoptico-intermedius takes
two different courses, just as shown in carp by SHELDON : the one
of which runs paralled to the recessus preopticus, or the tractus
preoptico-intermedius pars medialis, and the other runs along the outer
margin of the fasciculus lateralis hemisphaerii, or the tractus preoptico-
intermedius par lateralis.

(i) Tractus preoptico-intermedius pars medialis (SHELDON, '12).

The tractus preoptico-intermedius pars medialis descends from the
nucleus preopticus to the nucleus intermedius parallel to the recessus
preopticus and to the median cavity, and passes by the fasciculus
medialis hemisphaerii (Text-fig. 26). It is said by SHELDON that this
tract is composed of the double tracts of ascending (tractus preoptico-
intermedius pars medialis) and of descending (tractus intermedio-
preopticus pars medialis) fibers.

(ii) Tractus preoptico-intermedius pars lateralis (SHELDON, '12).

This fine fiber tract runs along the lateral side of the fasciculus
lateralis hemisphaerii towards the nucleus intermedius, starting from
the nucleus preopticus (Text-fig. 26).

(f) Some fibers which enter the tractus olfacto-habenularis.

(i) Fibers which enter the tractus olfacto-habenularis from the nucleus
preopticus.

Those fibers, which are non-medullated, combine with the tractus
olfacto-habenularis at the latero-dorsal portion of the fasciculus medialis
hemisphaerii.

This group of fibers may probably belong to the tractus preoptico-
habenularis pars anterior, tractus preoptico-habenularis pars medialis,
and tractus preoptico-habenularis pars lateralis named by SHELDON
('12). SHELDON describes that the pars anterior originates from the
nucleus parvocellularis strati grisei, and both the pars medialis and
pars lateralis originate from the nucleus magnocellularis strati grisei.
Also, HOLMGREN ('20) found the tractus preoptico-habenularis pars
lateralis at the same portion, which we have traced, and moreover,
he states regarding this tract as follows : " Bei *Osmerus* habe ich
auch ein ähnliches Bündel gesehen, aber dieses hat eine ganz andere
Bedeutung. .Es handelt sich hier nämlich um einem Opticusbündel

(Tractus optico-habenularis?), das durch eigentümlichen Verlauf sehr leicht für ein Preopticusbündel genommen werden kann. Das Bündel liegt in der Transversalebene der basalen Wurzel des Opticus an der basalen Wurzel vorüber zieht, trennt er einige Fasern ab, welche in das Telencephalon medium hineindringen. Diese erstrecken sich fächerförmig divergierend sogar bis zum Lateralwand des Nucleus preopticus, hier biegen sie aber gerade um und ziehen denselben Weg zurück....."
If the fact is constant in teleosts that the nucleus preopticus is directly related with the optic bundle, it is worthy of investigation.
(ii) Although I perceived many more fibers arising from the nucleus entopeduncularis, nucleus intermedius, etc., I was unable to trace their ultimate ending in the present study.

palaeostr — tr. mediano. preopt.
corp. precomm — tr. strio. thal. cruc.
— tr. strio. thal. incruc.
uucl. olf. lat· — tr. preopt. intermed.
nucl. preopt· — tr. hyp. olf. med.
— tr. olf. thal. med.
— tr. teniae.
n. opt — tr. preopt. hab.
habenula — tr. strio. thal. cruc.
— tr. strio. thal. incruc.
— tr. olf. thal. lat.

fasc. med. hem.   fasc. lat. hem.
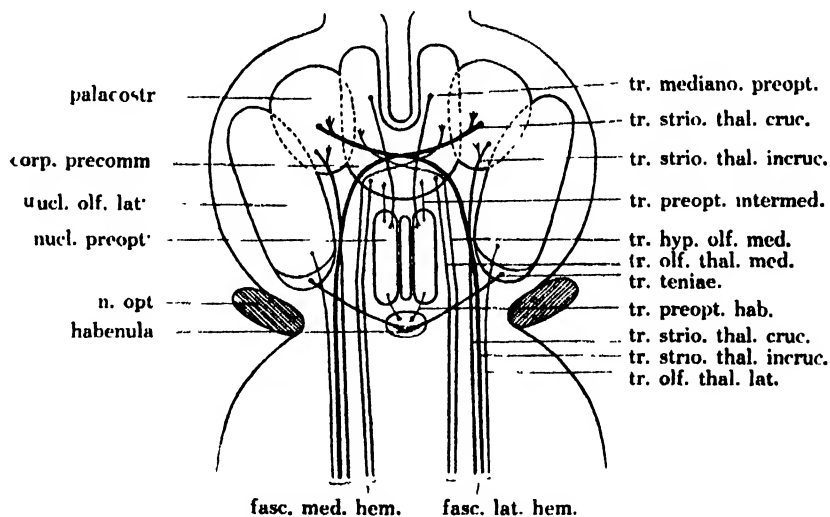
Text-fig. 33.   Horizontal scheme of the diencephalic connecting fibers.

III. GENERAL REMARKS.

The teleostean brains have been studied already by many neurologists, especially by L. EDINGER ('88, '92), L. STIEDA ('68, '73), C. L. HERRICK ('91, '92), B. HALLER ('98), E. H. CATOIS ('02), K. GOLDSTEIN ('05), A. KAPPERS ('06, '07, '12), J. B. JOHNSTON ('11), R. E. SHELDON ('08, '09, '12) and N. HOLMGREN ('18, '20), using many different species of teleosts, such as *Anguilla, Barbus, Cyprinus,*

Gadus, Lophius, Pleuronectus, Salmo, Sirulus, Thynus, etc.

As I have already stated, so far as the nuclei and fiber tracts of the cerebrum are concerned, *Carassius auratus* (L.) closely resembles the carp, and unless otherwise noted the statement made by SHELDON may be taken as also applicable to *Carassius auratus* (L.). In the following, I have enumerated some more or less noticeable differences found in *Carassius auratus* from the carp.

(1) In *Carassius auratus* (L.), the fissures which run above the cerebral hemispheres are not so distinct as in carp.

(2) The differentiation of the nucleus pyriformis and the nucleus olfactorius lateralis is more difficult in *Carassius auratus* (L.) than in carp.

(3) At the rostral portion of the basal lobe, the tractus olfactorius lateralis is faintly differentiated as compared with carp.

(4) The most medial part (pars medialis?) of the tractus olfactorius lateralis probably decussates in the anterior commissure, while GOLD-STEIN notes the decussation and SHELDON the non-decussation in the carp.

(5) At the rostral end of the basal lobe, there is no distinction between the tractus olfactorius ascendens and the tractus olfactorius medialis pars lateralis within the tractus olfactorius medialis, as is shown in carp.

(6) The fibers of the tractus olfactorius medialis pars lateralis do not end in the nucleus olfactorius dorsalis.

(7) Some fibers of the commissura olfactoria interbulbaris end into the nucleus medialis and pars commissuralis of the corpus precommissurale without decussation.

(8) Almost all of the fibers of the commissura olfactoria internuclearis are composed of medullated fibers.

(9) In regard to the fasciculus medialis hemisphaerii, only the two tracts, tractus hypothalamo-olfactorius medialis and tractus olfacto-thalamicus medialis, are traced.

(10) The fibers of the tractus olfacto-thalamicus medialis, slightly caudal to the anterior commissure, do not appear to increase in size.

(11) The tractus olfacto-hypothalamicus lateralis is not so distinct as in carp.

(12) The fibers of the tractus strio-thalamicus end in a smaller area

of the hypothalamus than in carp.

(13) The fibers of the tractus teniae are distinctly more medullated than those of carp.

The writer wishes here to express his deep graditude to Prof. S. HATAI for his helpful direction, and to Prof. S. HÔZAWA who did much to aid in the course of the work.

LITERATURE CITED.

1) BROOKOVER, C., 1910. The olfactory nerve, the nervus terminalis and the preoptic sympathetic system in *Amia calva*, L. Jour. Comp. Neur., Vol. 20, pp. 49–118.

2) CATOIS, E. H., 1902. Recherches sur l'histologie et l'anatomie microscopique de l'encephale chez les poissons. Bull. scientifique de la France et de la Belgique, Tome 36, pp. 1-166.

3) EDINGER, L., 1888. Untersuchungen über die vergleichende Anatomie des Gehirns. I. Das Vorderhirn. Abhdlg. d. Senckenberg. Naturf. Gesellsch., Bd. 15, pp. 90-122.

4) EDINGER, L., 1892. Untersuchungen über die vergleichende Anatomie des Gehirns. II. Das Zwischenhirn. Ibid., Bd. 18, pp. 1–55.

5) GOLDSTEIN, K., 1905. Untersuchungen über das Vorderhirn und Zwischenhirn einiger Knochenfische. Arch. f. mikr. Anat. u. Entw., Bd. 66, pp. 145-219.

6) HALLER, B., 1898. Vom Bau des Wirbelthiergehirns I. Theil. *Salmo* und *Scyllium*. Morph. Jahrb., Bd. 26, pp. 345-641.

7) HERRICK, C. L., 1891 a. The commissures and histology of the teleost brain. Anat. Anz., Bd. 6, pp. 676-681

8) HERRICK, C. L., 1891 b. Contributions to the morphology of the brains of the bony fishes. II. Studies on the brains of some American fresh-water fishes. A. Topography. Jour. Comp. Neur., Vol. 1, pp. 228-245.

9) HERRICK, C. L., 1891 c. Contributions to the morphology of the brains of the bony fishes. II. Studies on the brains of some American fresh-water fishes. (Continued.) B. Histology of the rhinencephalon and procencephalon. Ibid., pp. 333-358.

10) HERRICK, C. L., 1892 a. Contributions to the morphology of the brains of the bony fishes. II. Studies on the brains of some American fresh-water fishes. (Continued.) C. Histology of the diencephalon and mesencepha'on. Ibid. Vol. 2, pp. 21-72.

11) HERRICK, C. L., 1892 b. Additional notes on the teleost brain. Anat. Anz., Bd. 7, pp. 422-431.

12) HOLMGREN, N., 1918. Zur Kenntniss des Nervus terminalis bei Teleostieren. Folia Neuro-Biologica, Bd. 11, pp. 16-36.

13) HOLMGREN, N., 1920. Zur Anatomie und Histologie des Vorder- und Zwischenhirns der Knochenfische. Acta Zoologica., Bd. 1, pp. 137-315.

14) JOHNSTON, J. B., 1911. The telencephalon of Ganoids and teleosts. Jour. Comp. Neur., Vol. 21, pp. 489-591.

15) KAPPERS, A., 1906. The structure of the teleostean and selachian brain. Jour. Comp. Neur., Vol. 16. pp. 1-112.

16) KAPPERS, A., 1912. Die Furchen am Vorderhirn einiger Teleostier Anat. Anz, Bd. 40, pp. 1-18.

17) LISSNER, H., 1924. Das Gehirn der Knochenfischen. Wissenschaftl. Meeruntersuch. (Kiel), Bd. 15, pp. 123-188.

18) SHELDON, R. E., 1908. An analysis of the olfactory paths and centers in fishes. Anat. Rec., Vol. 2, pp. 108-109.

19) SHELDON, R. E., 1909. The nervus terminalis in the carp. Jour Comp Neur., Vol. 19, pp 191-201.

20) SHELDON, R. E,, 1912. The olfactory tracts and centers in teleosts. Jou. Comp. Neur., Vol. 22, pp. 176-339.

21) SHELDON, R. E., 1914. Paraffiine-WEIGERT methods for the staining of nervous tissue, with some new modifications. Folia Neuro-Biologica, Bd. 8, pp. 1-28

22) SHELDON, R. E. and BROOKOVER, C., 1909. The nervus terminalis in teleosts Anat Rec., Vol. 3, pp. 257-259.

23) SPIELMEYER, W., 1924. Technik der mikroskopischen Untersuchung des Nerven-systems. Berlin.

EXPLANATION OF FIGURES.

Photomicrographs were taken from preparations made by the toluidin blue method, and show the arrangement of nuclei of the cerebrum.

PLATE XX

Fig. 1. Dorsal aspect of the brain of *Carassius auratus* (L.). (×2).

Fig. 2. Transection through the rostral portion of the cerebrum The level is the same as that of text-fig. 14. (×30).

Fig. 3. Transection through the slightly rostral portion of the anterior commissure of the cerebrum. (×30).

Fig 4. Transection through the anterior portion of the anterior commissure of the cerebrum. (×30).

PLATE XXI

Fig. 5. Transection through the posterior portion of the anterior commissure of the cerebrum. (×30).

Fig. 6. Transection at the level of the recessus preopticus of the cerebrum (×30).

bu. bulbus olfactorius.

tr. tractus olfactorius.

cb. cerebrum.

zb. mesencephalon.
cr. cerebellum.
vg. lobus vagus.
sp. medulla spinalis.
 a. nucleus olfactorius dorsalis (primordium hippocampi of SHELDON).
 b. palaeostriatum.
cm. nucleus medianus of corpus precommissurale.
 d. nucleus olfactorius lateralis.
vm. ventriculus medialis.
tm. tractus olfactorius medialis.
 e. nucleus teniae.
cp. pars supracommissuralis of corpus precommissurale.
cc. pars commissuralis of corpus precommissurale.
 f. nucleus commissuralis lateralis.
no. nervus opticus.
ac. commissura anterior.
ci. nucleus intermedius of corpus precommissurale.
 g. nucleus entopeduncularis.
fl. fasciculus lateralis hemisphaerii.
hp. nucleus parvocellularis strati grisei.
hm. nucleus magnocellularis strati grisei.
rp. recessus preopticus.

.
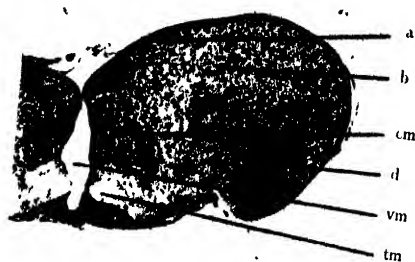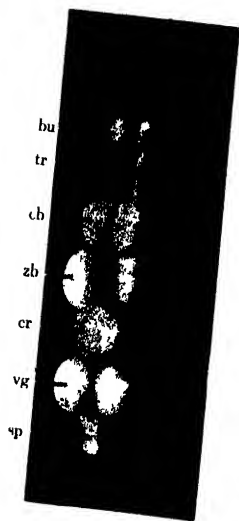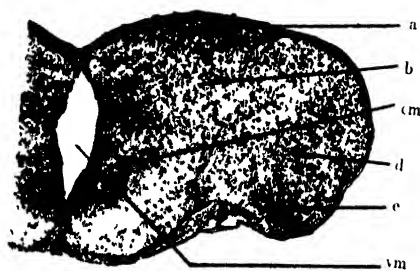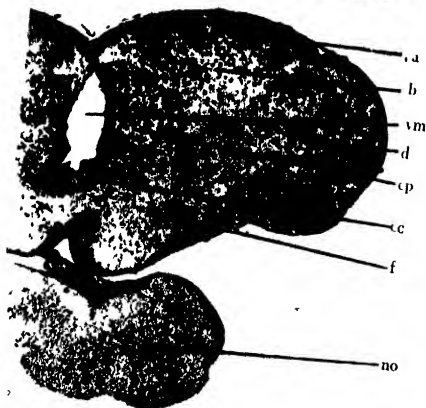
H. TUGE: Fiber tracts of the cerebrum of *Carassius auratus* (L.).

Fig. 5.

Fig. 6.

H. Tuge : Fiber tracts of the cerebrum of *Carassius auratus* (L.).

-
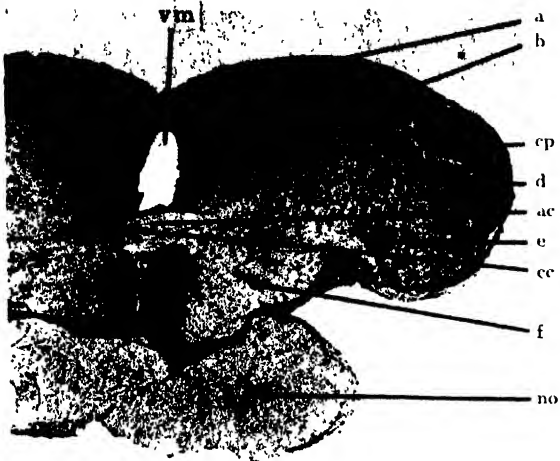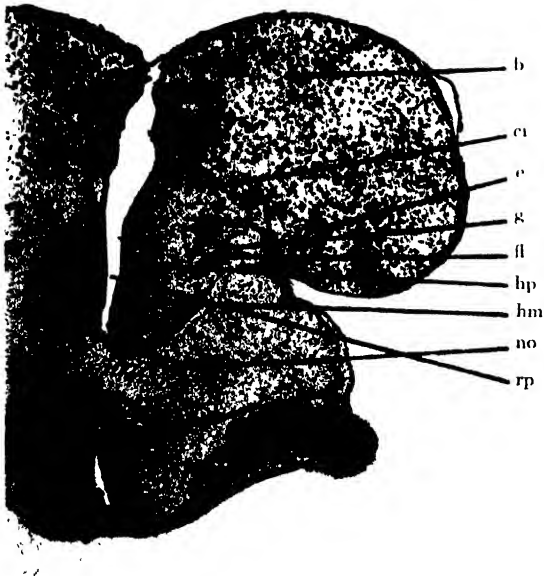
Reactions to Centrifugal Force in the Holothurian, *Caudina chilensis* (J. MÜLLER).[1]

By

TOSHIHIKO YAMANOUCHI.

(Zoological Laboratory, College of Science, Kyôto Imperial University.)

I. INTRODUCTION.

The reactions of Echinoderms to gravitational force are a long-established problem in animal Behaviorism since the observation of ROMANES (1885) on the righting reaction of a starfish and the experiment of LOEB (1891) in the geotropism of *Cucumaria*. Yet the receptive mechanism for gravitational force remains unsolved in many animals up to the present day, except for the cases of animals which posses statocysts. Several factors affecting the righting reaction were found and interpreted in detail recently by PARKER (1922), WOLF (1925) and by FRÄNKEL (1928); however, the true receptor in the righting reaction is yet obscure.

As for geotropism, the problem remains unsolved. In three marine animals (*Arenicola, Branchiomma, Convoluta*), v. BUDDENBROCK (1912, 1913, 1924 p. 106) proved experimentally, by extirpation of it, that it is really a statocyst which initiates the geotropic response. But geostropism (or geotoxis) and righting reaction are known in animals possessing no statocysts. It is, moreover, a highly interesting fact that an isolated portion of the body can respond to gravitation as first observed in the righting reaction of starfish by ROMANES (1885) and later confirmed by WOLF (1925), and in *Ophioderma* by MANGOLD (1909), secondly in the case of negative geotropism in *Asterias* and *Panceri* by MANGOLD (1909) and in the tail of *Caudina* by YAMA-NOUCHI (1929, TABLE 8).

LOEB (1891) first used the centrifugal machine for the study of the negative geotropism in *Cucumaria*. Recently, FRÄNKEL (1927 a,

---

[1] A Contribution from the Marine Biological Station, Asamushi, Aomori-Ken.

b) proved the negative geotropism in *Littorina* and *Solen* by the use of a turn-table.

In *Caudina chilensis*, the positive geotropism of the anterior portion of the trunk (YAMANOUCHI 1926) and the negative geotropism of the tail (YAMANOUCHI 1929) were investigated in detail. In the present investigation these above mentioned tropisms are experimentally proved by the use of a turn-table, as adopted by FRÄNKEL in the study of *Solen* (FRÄNKEL 1927 b).

The investigations were carried on in the Laboratory of the Asamushi Marine Biological Station in September 1928. I desire to express my sincere thanks to Prof. HATAI for his kind guidance and encouragement and also to Assist. Prof. KOKUBO and Assist. TAMURA for their warm friendship during my stay at Asamushi.

II. METHOD.

The centrifugal machine used in this experiment is shown in Fig. 1, with its dimensions. The rotating disk consists of a shallow circular metal vessel as an electro-phorus filled with a mixture of beeswax and paraffine. The lower flat end of the axis is laid on a steel ball for the purpose of minimizing friction and of making the pivot withstand heavy weight. (I thank my friend Mr. TSUCHIYA for his kind advice in constructing this apparatus).

The turn-table was driven by an a. c. motor of 1/12 H. P. using the lighting circuit. The rotating velocity was measured accurately either by eye or by a trochometer.

The large glass jar was 28 cms. in its inner diameter and 23 cms. in height. This was laid on the turn-table and was filled with a layer of fine sand 10 cms. deep, which was brought from the dwelling place of *Caudina*, and with sea water up to a depth of 3 cms. over the sand.

In the sand of this jar, the animal was imbedded at a position 10–12.5 cms. away from the center and a few cms. above the bottom, in such a manner that the longitudinal main axis of the body was placed parallel both to the bottom and to the glass wall of the jar (Fig. 1, 2). The glass jar containing the animal was rotated, and the responses of the tail and the trunk were observed after the rotation. The

duration of rotation was in most cases 15 minutes, and in a few cases 20 minutes. The isolated tail was also fixed with a pin to the wax of the turn table at various distances from the center, and was made to revolve.

Caudina used in this experiment were all adult individuals in their appearance. They were collected with great care in the day time and were preserved in the sand of an aquarium up to the time of the experiment, which was carried on during the night of the same day.
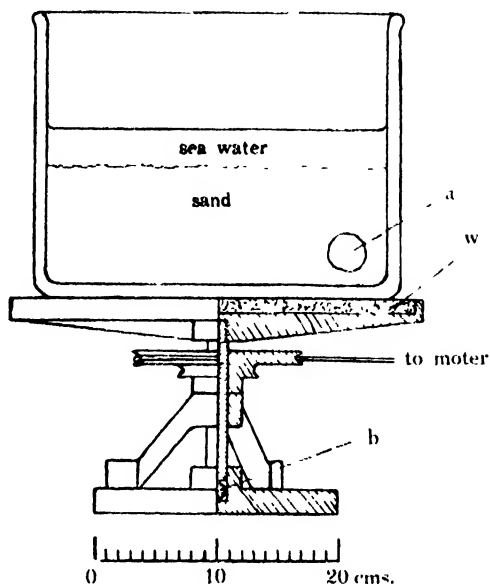

Fig. 1.   The turn table, on which a glass jar is laid.

a.   *Caudina*, imbedded in the sand
w.   Mixture of beeswax and paraffine.
b.   Steel ball.

III.   REACTION.

a) *The intact animal.*   As a preliminary experiment, the animal was imbedded in the sand of glass jar in various positions, and the responses were observed after the rotation, and it finally proved to be most statisfactory for the animal to be imbedded in such a manner as is shown in a and a′ of Fig. 2.   As soon as the turn-table had come to rest after the rotation of from 15 to 20 minutes, the sand was carefully dug out and the position of the end of the tail and the form of the anterior portion of the trunk were confirmed.

In general, the anterior part bends in an outward direction from the center of rotation and more or less downward (b, b′ in Fig. 2). The amount of its reaction was much less than that of the tail.   The tail usually responded in the same manner as the trunk, but in the opposite direction.   After) the rotation. the tip end was found in a

more upper and more inner position than the initial situation (b, b′ in Fig. 2). Sometimes, the tip end emerged on the surface of the
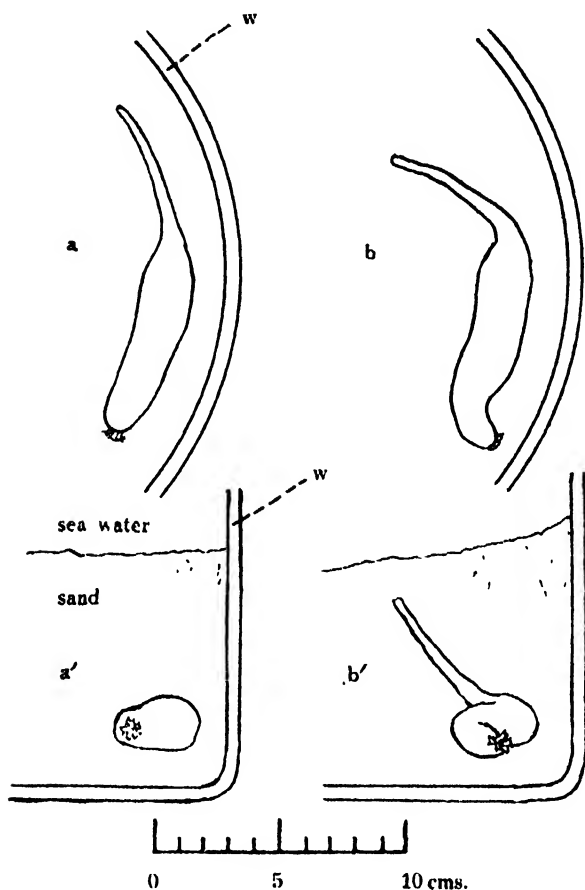

Fig. 2. Responses of the animal to centrifugal force. a-a′ and b-b′ are respectively the situations of *Caudina* before and after the rotation. a and b are horizontal views. a′ and b′ are elevations. w is a glass wall of the jar.

sand after the rotation. These facts indicate without doubt that the tail reacted negativegeotropically to both the gravitation and the centrifugal force.

The results of experiments are shown in TABLE 1. The direction of the animal in the revolution seems to be of no consequence

TABLE 1.

Reactions to centrifugal force.

The anterior part of the trunk bends outward and the tail bends inward. The duration of rotation was 15 minutes. Experiment made in September 1928.

t, temperature of the room in °C.
No, number of specimen.
d, distance of animal from the rotating axis in cms.
n, number of rotations per minute.

Date	t.	No.	d.	n.	Distance responded, in cms.		Direction of the revolution of animal
					Anterior part of the trunk (outward)	Tip end of the tail (inward)	
6	23	29	11	100	—	5	anterior
		35	12.5	130	—	2	,,
		37	,,	115	outward	2.5	,,
		40	12	120	outward	4	,,
		41	,,	117	outward	5	,,
		42	12.5	113	—	no reaction	,,
8	21.5	44	10	120	–	2	,,
		45	12.5	115	outward	0 -1	,,
		47	,,	,,	—	no reaction	,,
		48	,,	,,	—	no reaction	,,
		49	,,	,,	—	2	posterior
		50	,,	,,	—	no reaction	,,
		51	,,	120	—	0–1	,,
		52	,,	,,	outward	no reaction	,,
		54	,,	125	–	no reaction	,,
		55	11	,,	outward	1	,,
		56	11.5	,,	outward	0–1	,,
		57	11	,,	outward	no reaction	,,
		58	12.5	,,	no reaction	no reaction	,,
		59	,,	120	no reaction	1	,,
		60	,,	,,	—	1	,,
9	22	61	,,	81	—	1.5	,,
		62	,,	,,	—	1	ant.
		63	,,	78	outward	1	post
		64	,,	,,	outward	3	ant.
		65	10	102	—	no reaction	post.
		66	,,	,,	—	1	ant.

| Date | t. | No. | d. | n. | Distance responded, in cms. | | Direction of the revolution of animal |
					Anterior part of the trunk (outward)	Tip end of the tail (inward)	
		67	12.5	100	—	1	post.
		68	,,	,,	—	no reaction	ant.
		69	,,	113	—	3	post.
		70	,,	,,	—	6	ant.
		71	,,	120	outward	no reaction	post.
		72	,,	,,	—	1.5	ant.
		73*	11	104	—	5	post.
		74*	10	,,	—	3	ant.
10	22	75*	12.5	102	outward	1	post.
		76*	,,	,,	no reaction	no reaction	,,
	23	94	10	107	no reaction	7.5	ant.
		97	,,	113	—	3	,,

*The duration of rotation was 20 minutes.

(compare the last three colomns in TABLE 1).

The anterior end of the trunk is the most receptive portion for mechanical stimuli, especially when the whole body of the animal is

TABLE   2.

Arranged from TABLE 1.

	Distance reacted, in cms.	Number of cases	Reaction
Anterior part of the trunk	0	4	no reaction cases
	outward	12	reactive cases
Tip end of the tail	0	12	no reaction cases
	0–1	3	slightly reactive cases
	1 1.5 2 2.5 3 4 5 6 7.5	8 2 3 1 4 1 3 1 1	24 reactive cases

disturbed (YAMANOUCHI 1929 p. 82). So it often happened that the anterior part had retracted when I dug out the sand to ascertain the reaction of that part. Such doubtful cases were omitted in TABLE 1.

Summerizing the result shown in TABLE 1, TABLE 2 was formed. From TABLE 2, it is clear that both ends of the body of *Caudina* responded to centrifugal force as well as it had reacted to gravitation (YAMANOUCHI 1926, 1929).

b) The animal without nerve ring. The anterior-most portion of the body, which includes the nerve ring, was destroyed either by hot sea water (it was reported by me in 1929 that *Caudina* loses vitality at a temperature above 39°C.) or by amputation of that portion. It is needless to say that the anterior part thus treated showed no sign

TABLE 3.

Animals without nerve ring.

A. About one centimeter of the anterior end of the body was immersed in hot sea water and was coagulated.

B. The same portion was tightened firmly by a silk-thread and the anterior part was cut off.

The duration of rotation was 15 minutes. Distance of the animal from the rotating axis was 11.5 cms.

	Date	t.	No.	Number of rotations, per minute	Reaction of the tail
	10	22	78	107	2 cms inward
			79	107	2 cms inward
			83	107	2.5 cms inward
A			84	107	2 cms inward
			92	107	no reaction
			93	107	3.5 cms inward
			95	113	no reaction
	10	22	80	107	4 cms inward
			81	107	7 cms inward
			86	104	1.5 cms inward
B			87	104	4 cms inward
			89	106	2.5 cms inward
			90	106	6 cms inward
			96	113	3 cms inward

of response to centrifugal force. The tail of *Caudina*, whose nerve ring had been removed, was able to respond just as well as in the intact animal (TABLES 2, 4).

TABLE 4.

Arranged from TABLE 3.

Distance reacted, in cms.	Number of cases	Reaction
0	2	no reaction cases
1.5	1	
2	3	
2.5	1	
3	1	12 reactive cases
3.5	1	
4	3	
6	1	
7	1	

c) Experiments for control. During the rotation, the surface of sea water and the sand become conical in form. When the rotation ceases, the sand does not assume the initial appearance, but the surface of it is higher to some extent along the margin than in the central part. In such a case, the movement of sand particles to the center of rotation is as follows:

(1) When the turn-table is set in motion, they migrate outward,

(2) they remain at a definite distance from the center, when the rotation becomes uniform (ordinarily after a few seconds),

(3) and they migrate inward, when the rotation becomes slow and ceases.

As the movement of the sand particles is not reversible, judging from the surface of the sand after the rotation, they are driven somewhat outward by the rotation of 15–20 minutes interval.

So it is reasonably considered that the body of the animal makes, as a whole, an outward translation motion by the rotation. Next, the turn-table made perfectly smooth rotations when it was not loaded, but in the case when it was loaded with the heavy jar, it vibrated considerably because of the ununiform distribution of the weight along the rotating axis. It is also suspected that the trunk and the tail

would be differently affected directly by sand particles. The above stated conditions, however, seem in no way to interfere with the experimental results, for the following experiments for control prove it (TABLE 5).

TABLE 5.

Animals killed by hot sea water.

In any case, there was no change of the form, nor of the position in the body of the dead animals.

Date	t.	No.	Number of rotations, per minute	Temperature in C at which the animal was killed.
6	23	30	110	60
		31	140	46
		34	130	73
		36	115–130	51
		38	117	60
		39	130	58
		43	100	70
8	21.5	46	115	57
10	22	85	107	55
		88	104	58
		91	106	52

. The heat coagulation of the body wall of *Caudina* is observed by TAO (1927) and YAMANOUCHI (1929). When the animal is immersed in hot sea water, the hardness of the body wall is dependent upon the temperature of water. At high temperatures (above 55-60°C.) the body wall becomes hard and stiff due to the heat coagulation of the tegument (TAO, 1927 p. 272); at considerably low temperatures (between 40 and 55°C.) it is soft. Varying the temperature of sea water, we have different degrees of hardness of the body wall.

Such a dead animal was imbedded in the sand as in the ordinary case. The only difference was the position of the tail, the end of which was raised about 2 to 3 centimetres from the horizontal position of the trunk. There was exhibited no sign of transposition in any part of the body, nor any change of the body form by the rotation of 15 minutes interval. A gummi-tubing which had 3.5 mms. outer diameter was placed in the sand at the same position where *Caudina* had been imbedded, and was rotated just the same as in the ordinary experiments; after a rotation of 15 minutes, no remarkable transposition

of the above tubing was found. Such a result was, also, obtained in a case when the same was tried with a small pencil.

It is, therefore, natural to consider that the reactions shown in TABLES 1, 2, 3 and 4 are true responses of *Caudina* to external forces and not the result of passively and mechanically driven motions.

d) The isolated tail. The basal part of the isolated tail was fastened to a lead block and this was imbedded in the jar. After the rotation, the isolated tail remained in contraction and remarkable changes were not observed at all.

The isolated tail was, also, fastened on the wax (w in Fig. 2) of the rotating disk by a pin. The turn-table was rotated in the air. Several experiments (using 13 isolated tails) were tried at various intervals (from 30 seconds to 5 minutes) and at various velocities (from 60 to 207 rotations per minute). Throughout the experiments the tails remained always in tonic contraction. The remaining part of the tail, except the pinned part, was either in most cases directed outward from the rotating axis, or in a fewer cases remained in severe contraction showing no outwardly directed bending.

Non-reactiveness of the isolated tail in the rotating experiments seems to be due to the severely shortened state of the muscles and to the other unnatural conditions (for instance, the pinning of the basal part).

IV. REMARKS.

LOEB (1891, p. 181) tried a rotating experiment with *Cucumaria cucumis*, but he could obtain no result, for the animal remained motionless during the rotation. Such was also true of *Caudina*, *when* it was rotated in sea water. In these cases there is no means to detect the reactions, for the centrifugal force disappears when the rotation ceases. In such animals as *Littorina*, *Solen* and *Caudina*, the responses remain without much alternation after the rotation.

In this and FRÄNKEL's experiments (1927 b, p. 205), the magnitude of reactions were measured after the turn-table had stood still, so it must be remembered that the magnitude measured is to some extent relative to the state of sand particles. In FRÄNKEL's data (1927 b, p. 208) and in this (TABLES 1, 3) there is considerable number of

no reaction cases. The cause of this occurrence will, in part, be attributed to the change of the experimental conditions from the natural. FRÄNKEL well said that — die Gültigkeit des Resultantgesetzes wenigstens qualitative erweisen ist (1927 b, p. 208). The same is true in this investigation.

V. SUMMARY.

1. When *Caudina* is imbedded in sand and is rotated, it responds to centrifugal force in such a manner that the anteriormost part of the trunk reacts in a positive geotropic sense and the tail in a negative geotropic sense (TABLES 1, 2).

2. Even when the anterior part of the body including the nerve ring is destroyed either by heat or by amputation of the part, the tail reacts to the centrifugal force just as the intact animal will (TABLES 3, 4).

VI. LITERATURE CITED.

1) BUDDENBROCK, W. von. 1912. Über die Funktion der Statozysten im Sand grabender Meerestiere (*Arenicola* u. *Synapta*). Biol. Zentralbl. Bd 32, p 564, and in Zool. Jahrb., Abt. Allg. Zool u. Physiol., Bd. 33, p. 441, 1913.

2) BUDDENBROCK, W. von. 1924. Grundriss der vergleichenden Physiologie. I. Teil: Sinnesorgane und Nervensystem. Berlin. p. 106.

3) FRÄNKEL, G. 1927 a. Beiträge zur Geotaxis und Phototaxis von *Littorina*. Zeit. f. vergl Physiol., Bd. 5, pp. 585–597.

4) FRÄNEEL, G. 1927 b. Die Grabbewegung der Soleiden Zeit. f. vergl. Physiol., Bd. 6, pp. 205, 208.

5) FRÄNKEL, G. 1928. Über den Auslösungsreiz des Umdrehreflexes bei Seesternen und Schlangensternen. Zeit. f. vergl. Physiol, Bd. 7, p. 365.

6) LOEB, J. 1891. Über Geotropismus bei Thieren. PFLÜGER's Arch., Bd. 49, p. 181.

7) MANGOLD, E. 1909. Sinnesphysiologische Studien an Echinodermen. Zeit. allg. Physiol., Bd. 9, p. 142.

8) PARKER, G. H. 1922. The Geotropism of the Seaurchin *Centrechinus*. Biol. Bull., Vol. 13, pp. 374–383.

9) ROMANES, G. J. 1885. Jelly-Fish, Star-Fish and Sea-Urchins. London. (Cited from BAGLIONI in WINTERSTEIN's Handb. d. vergl. Physiol., Bd. 4, pp. 147-150).

10) TAO, Lieh. 1927. Physiological Characteristics of *Caudina* Muscle, with some Accounts on the Innervation. Science Reports, Tôhoku Imp. Univ., 4th Series (Biology), Vol. 2, p. 272.

11) WOLF, E. 1925. Physiologische Untersuchung über das Umdrehen der Seesterne und Schangensterne. Zeit. f. vergl. Physiol., Bd. 3, pp. 209–224.

12) YAMANOUCHI, T. 1926.   Some preliminary Notes on the Behavior of the Holoturian,
        Caudina chilensis (J. MÜLLER).   Science Reports, Tôhoku Imp. Univ., 4th
        Series (Biology), Vol 2, pp. 85-91.

13) YAMANOUCHI, T. 1929.   Notes on the Behavior of the Holothurian, *Caudina chilensis*
        (J. MÜLLER).   Science Reports, Tôhoku Imp. Univ., 4th Series (Biology),
        Vol. 4, pp. 82, 86, 107.

Fossile Koniferenhölzer aus Sendai-Tertiär. I.

Von

MASAHIKO TAKAMATSU.

(Biologisches Institut der Kaiserlichen Tôhoku Universität, Sendai).

Tertiäre Braunkohlenlager sind in der Nähe von Sendai weit ver-
breitet, und die Anzahl der Orte, wo sich stark gepresste Fossilhölzer
reichlich bilden, ist sehr gross.   Sie dienen hauptsächlich zu Feuerungs-
zwecken, und nur wenige, wohlerhaltene werden zu Holzschnitzarbeiten
verwandt.   Die in der Braunkohle erhaltenen Hölzer scheinen mir
vielen Arten anzugehören.   Aber die botanische Untersuchung dieser
Fossilblöcke ist bisher noch sehr dürftig.   Nur über eine einzige Art,
Sequoioxylon miyagiense, wurde vor kurzem von Fräulein YASUI
berichtet.

In vorliegender Mitteilung sollen einige bestimmbare Fossilholz-
Arten aus diesem Gebiet beschrieben werden.

Die Untersuchung wurde unter Leitung von Herrn Prof. Dr.
M. TAHARA ausgeführt.   Ich spreche ihm auch hier meinen herzlichsten
Dank für seine freundliche Führung und Kritik aus.   Ausserdem bin
ich auch den Herren S. ENDO und K. OHARA zu grossem Dank
verpflichtet, die bei der Bestimmung einiger Arten mir ihren wertvollen
Rat erteilt haben.

1. Taxodioxylon sequoianum (MERCKL.)
SCHMALH. erw. GOTH. em.

Die von mir untersuchten Braunkohlenhölzer wurden in der Nähe
der Otamaya-Brücke gesammelt.   Die Fossilien sind äusserst gut er-
halten.   Text-Fig. 1 gibt den Fundort wieder; die inmitten der schnel-
len Strömung stehenden zwei grossen Blöcke sind Überreste stehender
fossiler Koniferen-Stämme, und sie sollen hier behandelt werden.   Es
ist wahrscheinlich, dass sie seinerzeit dort gewachsen sind.   Diese
schwarz-braunen Blöcke sind von der gleichen Art und sind je 1 und
1,3 m im Durchmesser.   Das obere Ende der Stämme ist durch das
strömende Wasser weggerissen worden.

Querschnitt. Spätholzzone schmal, nur 2–5 Zellreihen dick, jede Zelle sehr dickwandig, tangential sich erstreckend. Die Zellen in der

Textfig. 1. *Taxodioxylon sequoianum* GOTH. im Fundorte.

Frühholzzone weitlumig, radial-länglich oder ganz viereckig (Text-Fig. 2). Übergang vom Frühholz zum Spätholz in einem Jahresringe sehr plötzlich. Traumatische Harzgänge häufig, stets sich ans Spätholz anschliessend. Die diese Harzgänge umgebende innerste Zellschicht besteht aus Parenchymzellen mit augenförmigen Poren, und diese Zellschicht ist wieder von einer oder einigen Querwände besitzenden Tracheidenschichten umgeben (Taf. XXII, Fig. 1). Markstrahlen geradlinig. Markstrahlen-Abstand 3–7 Tracheiden. Horizontalwände der Markstrahlen mit runden oder elliptischen Epiporen. Harzparenchym mit dunkel-braunen Substanzen, überall zerstreut, besonders im Spätholz reichlich.

Radialschnitt. Im Spätholz Tracheiden mit wenigen kleinen runden Tüpfeln mit schief-spaltfömigen Poren, dagegen im Frühholz die Tüpfel in den Tracheiden zahlreich, grösser, rund oder elliptisch mit runden oder elliptischen Poren; die Tüpfel ein- oder zweireihig (Taf. XXII, Fig. 2) und selten, 3–4 reihig, öfters ein wenig alternierend. SANIosche Streifen deutlich. Die Enden der Tracheidenwände in Radialrichtung schwach gekrümmt. Querwände des Harzparenchyms

dünn und glatt. Hoftüpfel auf den Seitenwänden der Markstrahlzellen epiporähnlich, schmal behöft, im Frühholz horizontal, aber im Spätholz etwas schräg, also echt taxodioid (Taf. XXII, Fig. 3 u. 4), in den mittleren Zellen ein- und in den Randzellen zweireihig; die Zahl der Tüpfel im Kreuzungsfeld 1–4 (selten 5) in den mittleren Zellen, aber 4–7 (selten 8) in den Randzellen; Tangentialwände dünn, glatt.
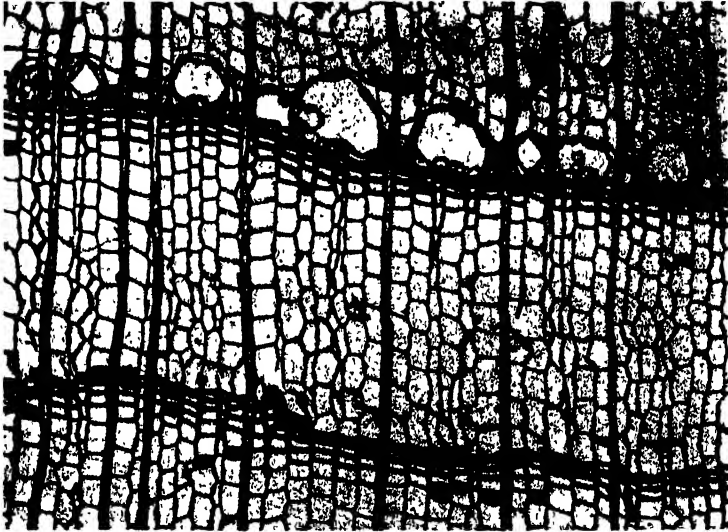
Textfig. 2. *Taxodioxylon sequoianum* GOTH.
Querschnitt des Holzes mit traumatischen Harzgänge.   × 100.

senkrecht, schief oder schwach gewölbt; Horizontalwände ziemlich dick, mit kleinen runden oder elliptischen Epiporen. (Text-Fig. 3, A).

Tangentialschnitt. Markstrahlen meistens einreihig, selten in ihren mittleren Partieen zweireihig, in 2–20 Stockwerken (meistens 2–3, äusserst selten 42); Die Zellen, weitlumig, rund oder elliptisch, dünnwandig. Tangentiale Hoftüpfel, in den Tracheiden im Frühholz vereinzelt, rund, ziemlich gross (Text-Fig. 3, B), im Spätholz zahlreich, klein, rund mit winzigen gekreuzten oder schräg laufenden Poren, ein- und nur sehr selten zweireihig (Taf. XXII, Fig. 5). Harzparenchym kurzzellig, Querwände glatt; tangentiale Tüpfel im Frühholz elliptisch mit schräg-elliptischen Poren. Querwände besitzende Tracheiden nicht selten sich unmittelbar ans Spätholz anschliessend, mit runden oder

elliptischen Hoftüpfeln auf den Radial-, Tangential- und Horizontal-
wänden (Taf. XXII, Fig. 6).
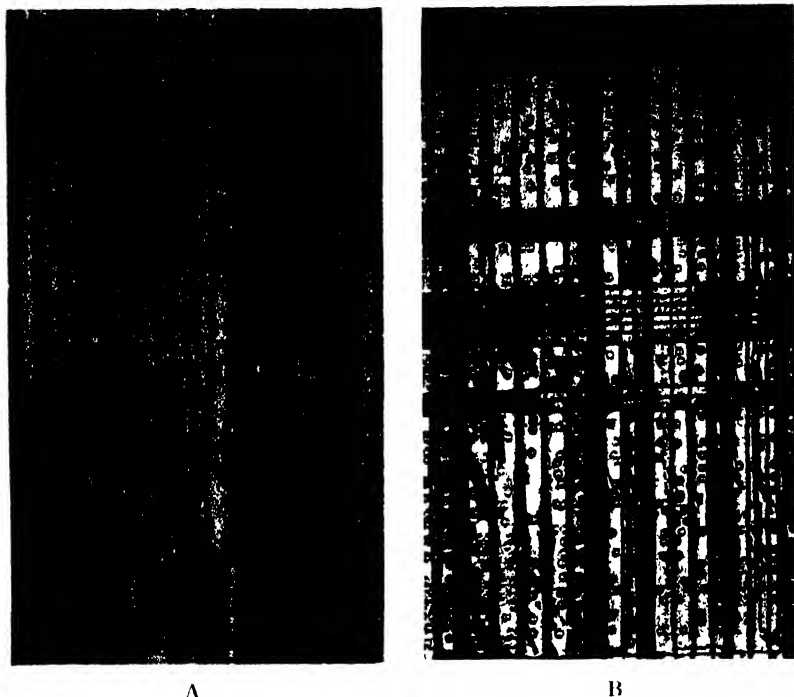
A   B

Textfig._3. *Taxodioxylon sequoianum* GOTH
A) Radialschnitt des Holzes   ✕ 40.
B) Tangentialschnitt des Frühholzes.   50.

Die obige Beschreibung stimmt mit der originalen von *Taxodioxylon
sequoianum* (MERCKL.) SCHMALH. erw. GOTH. em. sehr gut überein.
Die Zugehörigkeit meines Materials zu dieser Spezies kann also nicht
bezweifelt werden. Das Vorkommen von *Taxodioxylon sequoianum*
in Japan wurde zuerst 1926 von OHARA berichtet. Seine Beschreibung
der Struktur des Holzes weicht aber in einem einzigen Punkt vom
der originalen von GOTHAN ab. In OHARA's Material kommt nämlich
die cupressoid-taxodioide Markstrahlentüpfelung allgemein vor. Diese
Abweichung dürfte aber darauf beruhen, dass sein Material von einem
Zweig abstammte.

1917 beschrieb YASUI ein Koniferenfossil von Japan, mit Namen

Sequoioxylon hondoense YASUI. Die Struktur dieses Fossils hat Ähnlichkeit mit der des unsrigen. Aber durch die zweireihige Anordnung der Markstrahlentüpfel und die Abwesenheit der Tüpfelung in den Horizontalwänden der Markstrahlenzellen unterscheidet es sich deutlich vom unsrigen.

Erst kürzlich hat YASUI eine ausgedehnte Arbeit über die Struktur der japanischen Fossilhölzer publiziert. Dort finden wir eine neue Species *Sequoioxylon miyagiense* YASUI. Ihre Beschreibung dieses Koniferenfossils stimmt mit der unsrigen im grossen und ganzen gut überein. Betreffs der Tangentialtüpfelung der Tracheiden schreibt sie aber Folgendes: „ In the spring wood they (bordered pits) are found in the radial wall only." Also nach ihr scheinen bei ihrem Material die Tangentialtüpfel ganz auf das Spätholz beschränkt zu sein. Aber bei unserem Fossil kommen sie, wie oben erwähnt, auch beim Frühholz nicht so selten vor.

2. Taxodioxylon ishikuraense sp. nov.

Dieses Fossilholz, ein aus der Ishikura-Grube stammendes, grösseres Bruchstück, rührt wahrscheinlich von einem riesigen Stamm her. Es zeigt dunkel-braune Oberfläche und ist etwa 10 cm dick und 20 cm breit. Da die Erhaltung etwas schlecht ist, sind die Jahresringe mit freiem Auge nur undeutlich erkennbar.

Querschnitt. Unter dem Mikroskop Jahresring deutlich, Übergang aus Frühholz zu Spätholz allmählich (Taf. XXIII, Fig. 1). Tracheiden ziemlich zerbrochen, im Frühholz grösser, dünnwandig, radial-länglich, oder ganz viereckig, dagegen im Spätholz dickwandig, tangential länglich. Parenchymzellen zerstreut, besonderes im Spätholz zahlreich, sehr selten in Tangentialrichtung angeordnet.

Traumatische Harzgänge oft nur mitten im Frühholz vorkommend, stets tangential einreihig. Die diese Gänge umgebenden Partieen des Frühholzes stets gut erhalten, zahlreich im Harzparenchym (Text-Fig. 4). Die Markstrahlen-Abstände etwa 3—15 Tracheiden, und die Längen der Markstrahlenzellen 3–7 Tracheiden.

Radialschnitt. Inmitten des Frühholzes senkrechte, traumatische Harzgänge. Hoftüpfel auf den Tracheidenwänden gewöhnlich 1–2 reihig oder zerstreut, meistens rund, selten elliptisch, im Frühholz die

Poren schmal, schief, öfters gekreuzt, manchmal durch die äussersten
Ränder der Tüpfel zerrissen (Taf. XXIII, Fig. 5), im Spätholz die
Poren spaltförmig, schief, öfters gekreuzt, stark zerrissen. Markstrahlen
fast geradlinig ; die Horizontalwände ziemlich dick, mit etwas elliptischen
Epiporen ; Tangentialwände dünn, glatt, stehend, schräg oder etwas
gekrümmt, nicht zerrissen ; die Hoftüpfel auf den Seitenwänden ellip-
tisch, horizontal oder schräg orientiert, im Frühholz weit lensförmig,
die Poren horizontal, schwach behöft (taxodioid) (Taf. XXIII, Fig. 6),
im Spätholz die Poren schräg, spaltförmig, mit breiten Höfen (cupres-
soid) ; im Kreuzungsfeld meistens 2-4 (sehr selten 5), in den Randzellen
die Tüpfel oft horizontal, zweireihig.
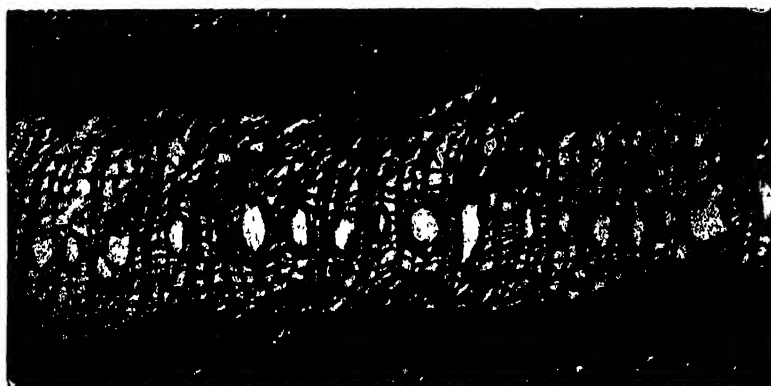
Textfig. 4. *Taxodioxylon ishikuraense* sp. nov.
Querschnitt des Holzes mit traumatischen Harzgange. × 70.

Tangentialschnitt. Tagentialhoftüpfel im Frühholz fehlend, aber
im Spätholz zerstreut, klein, rund; Markstrahlenzellen, länglich-elliptisch,
gewöhnlich in 2-13 Stockwerken (Taf. XXIII, Fig. 2) ; Querwände
der Parenchymzellen gewöhnlich schwach, sehr selten stark verdickt,
aber glatt, nicht getüpfelt.

Nach obiger Beschreibung ist der Bau unseres Fossils in vielen
Punkten dem der lebenden *Sequoia gigantea* TORR. sehr ähnlich. Aber
nach PENHALLOW ist der Übergang aus dem Frühholz zum Spätholz
bei *S. gigantea* gewöhnlich sehr plötzlich, und im Tangentialschnitt
sind die Markstrahlenzellen weitlumig, rund oder elliptisch, und ferner
sind die Markstrahlentüpfel ausschliesslich taxodioid. Solche Bildungen
finden sich bei unserem Fossil nie.

Die Tracheiden unsers Fossils haben keine spirale Verdickungen. Es hat also nichts zu thun mit der Struktur von *Taxus*. Aber die Gestaltung der Markstrahlen hat eine grosse Ähnlichkeit mit der dieser Gattung.

Cupressinoxylon wellingtonioides KRÄUSEL, das aus Schlesiens Tertiär in Deutschland stammt, zeigt mit unserem Material sehr nahe Verwandtschaft. Nach KRÄUSEL hat diese Art sehr nahe Beziehungen zu *Sequoia gigantea* TORR. Aber nach der Beschreibung von *C. wellingtonioides* sind die Markstrahlentüpfel nur echt cupressoid und auf dem Kreuzungsfeld in der Regel nur ein oder zwei, sehr selten drei. Dieser Aufbau zeigt deutliche Unterschiede zu unserem Material.

Diagnose. Traumatische Harzgänge nur inmitten Frühholz vorkommend, SANIOsche Streifen undeutlich, die Tüpfelporen auf den radialen Tracheidenwänden gekreuzt oder schräg, Übergang aus Frühholz zu Spätholz ziemlich allmählich, Markstrahlenbreite sehr schmal, die Zellen tangential-elliptisch, Tüpfelung auf den Seitenwänden der Markstrahlen im Spätholz cupressoid und im Frühholz taxodioid, Querwände des Harzparenchyms meistens glatt, selten schwach verdickt.

3. Cupressinoxylon Thujopsoides sp. nov.

Diese aus der Saiki-Grube gewonnene Fossilholz ist zum grossen Teil gut erhalten. Das Bruchstück ist 12 cm im Durchmesser und zeigt eine schwarz-braune Oberfläche. Die Jahresringe sind mit freiem Auge nur undeutlich sichtbar und ziemlich breit, was auf den Stamm schliessen lässt.

Querschnitt. Jahresringe deutlich, einige Frühhölzer schwach gedrückt, Übergang vom Frühholz zum Spätholz sehr allmählich (Taf. XXIV, Fig. 1). Tracheiden etwas gebrochen; im Frühholz ziemlich dünnwandig und gross, viereckig; im Spätholz häufig rund oder etwas tangential-länglich, sehr dickwandig. Harzparenchym nicht zahlreich, zerstreut, aber im Spätholz stellenweise in Tangentialreihe unregelmässig angeordnet. Markstrahlen-Abstände 4-15 Tracheiden. Markstrahlenzellen 2-5 Tracheiden lang, meistens harzhaltig.

Radialschnitt. Hoftüpfel der Tracheiden einreihig, meistens rund, in der Breite zwei Drittel der Tracheiden, im Frühholz die Poren lensförmig, horizontal oder selten schief, im Spätholz Hoftüpfel klein,

rund, mit fast stehenden, spaltförmigen Poren (Taf. XXIV, Fig. 5).
SANIOsche Streifen undeutlich. Markstrahlen fast geradlinig ; Tangen-
tialwände senkrecht oder etwas schräg, dünn und glatt, nicht getüpfelt ;
Horizontalwände sehr dick, mit wenigen Epiporen ; Hoftüpfel auf den
Seitenwänden, rundlich-elliptisch, die Poren spaltförmig, im Spätholz
schräg und im Frühholz mehr horizontal, in den mittleren Zellen
horizontal, einreihig und in Randzellen häufig zweireihig, im Kreuzungs-
feld 1–3 (meistens 2) in den mittleren Zellen, 2–4 in den Randzellen
(Taf. XXIV, Fig. 4).

Tangentialschnitt. Die Querwände des Harzparenchyms gewöhnlich
dünn, glatt oder selten schwach verdickt. Markstrahlen einreihig, in
1–9 (gewöhnlich 2–6) Stockwerken (Taf. XXIV, Fig. 2), alle Zellen
meistens rund oder ein wenig viereckig. Die Tangentialtüpfel auf den
Tracheidenwänden des Spätholzes rund, ziemlich gross, Poren schräg
oder horizontal, lensförmig (Taf. XXIV, Fig. 3).

Wie oben erwähnt, sind die Markstrahlen dieses Holzes echt
cupressoid. Also ist das Material sicher eine Art *Cupressinoxylon.*
Bei den lebenden Koniferen sind die cupressoide Markstrahlen besitz-
enden Hölzer ziemlich zahlreich. Aber unter ihnen zeigt *Thujopsis
dolabrata* in vielen Punkten engste Beziehungen zu unserem Fossil.
Indessen besitzt *Th. dolabrata* 1–5 Hoftüpfel im Kreuzungsfeld (KANE-
HIRA, FUJIOKA) und die Poren der Hoftüpfel auf den radialen Wänden
der Tracheiden sind meistens schräg und elliptisch.

In KRÄUSELs Monographie fossiler Koniferenhölzer findet man
keine der lebenden *Thujopsis dolabrata* entsprechende Art. Aber
Cupressinoxylon cupressoides KRÄUSEL und *C. Gothani* KRÄUSEL zeigen
mit unserem Fossil ziemlich nahe Verwandtschaft. Sie unterscheiden
sich von dem unsrigen in folgenden Punkten.

Bei *C. cupressoides* KRÄUSEL sind die Hoftüpfel auf dem Kreuzungs-
feld 1–2. Harzparenchymzellen reihen sich in Tangentialbändern und
die Hoftüpfel in den radialen Tracheiden sind sehr klein, etwa 1/3–
1/4 der Tracheidenbreite.

Bei *C. Gothani* sind die Querwände des Harzparenchyms dick und
glatt. Die Markstrahlentüpfel sind klein, meistens 1–2 im Kreuzungs-
feld. Tüpfel auf den Tangentialwänden der Tracheiden fehlen oder
sind höchstens sehr spärlich zerstreut.

Einige japanische Arten von *Cupressinoxylon* wurden schon von

verschiedenen Forschern mitgeteilt, nämlich *Cupressoxylon konzojiense* sp. prov. YASUI, *C. nagakudeénse* sp. prov. YASUI, *Cupressinoxylon Podocarpoides* REIS, *C.* sp. OHARA. Unter diesen Fossilien zeigt *Cupressoxylon nagakudeénse* ziemlich nahe Verwandtschaft. Aber bei dieser Art sind die SANIOschen Balken deutlich gestreift, und die Hoftüpfel auf den Markstrahlenseitenwänden sind gewöhnlich in zwei Reihen angeordnet. Ferner entwickelt sich kein Tüpfel auf den Markstrahlenquerwänden.

Diagnose. Übergang aus Frühholz zum Spätholz sehr allmählich, radiale Hoftüpfel im Frühholz zwei Drittel der Tracheidenbreite, die Poren meistens horizontal lensförmig, SANIOsche Streifen undeutlich, Hoftüpfel auf den Markstral-Seitenwänden echt cuppressoid, im Kreuzungsfeld 1–3, Querwände des Harzparenchyms gewöhnlich dünn und glatt, Tangentialtüpfel des Spätholzes ziemlich gross, die Poren horizontal, lensförmig.

FIGURENERKLÄRUNG ZU TAFEL XXII — XXIV

TAFEL XXII.   *Taxodioxylon sequoianum,* (MERCKL.)
SCHMALH. erw. GOTH. em.

Fig. 1. Querschnitt der traumatischen Harzgänge, ×350.
Fig. 2. Radialschnitt des Frühholzes, Tüpfelung an den Tracheidenenden, ×350.
Fig. 3 Radialschnitt des Markstrahls mit taxodioiden Poren, ×350.
Fig. 4. Radialschnitt des Markstrahls, etwas vergrössert, ×600.
Fig. 5. Tangentialschnitt des Spätholzes mit zahlreichen Hoftüpfeln, ×50.
Fig. 6. Tangentialschnitt der Querwand besitzenden Tracheiden, ×100.

TAFEL XXIII   *Taxodioxylon ishikuraense,* sp. nov.

Fig 1. Querschnitt des Holzes mit den Harzparenchymzellen, ×70.
Fig. 2. Tangentialschnitt des Spätholzes mit schmalen Markstrahlen, ×70.
Fig 3. Tangentialschnitt der traumatischen Harzgänge mit den Tüpfeln auf den tangentialen, radialen, und horizontalen Wänden, ×200
Fig. 4. Tangentialschnitt der unregelmässig verlaufenden, traumatischen Harzgänge, ×70.
Fig. 5. Radialschnitt des Frühholzes mit etwas zerrissenen Hoftüpfeln, ×350.
Fig. 6. Radialschnitt des Markstrahls mit taxodioiden Tüpfeln, ×500.

TAFEL XXIV.   *Cupressinoxylon Thujopsoides* sp. nov.

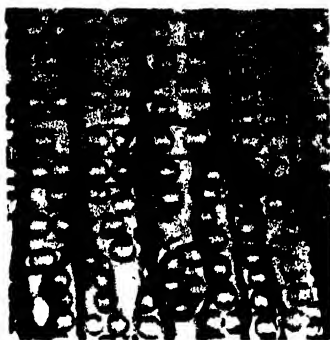Fig. 1. Querschnitt des dickwandigen Holzes, ×70.
Fig. 2. Tangetialschnitt des Spätholzes, ×70
Fig. 3. Tangetialschnitt des Spätholzes mit Hoftüpfeln, etwas vergrössert, ×350.
Fig. 4. Radialschnitt des Markstrahls mit cupressoiden Hoftüpfeln, ×200.
Fig. 5. Radialschnitt der Tracheiden mit fast horizontal elliptischen Poren, ×350.
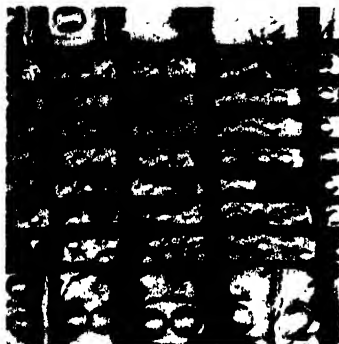
LITERATURVERZEICHNIS.

GOTHAN, W., 1905, Lebende und fossile Gymnospermenhölzer. Abhandl. Preuss. Geol. Landesanst., N. F., Bd. 45, pp. 1–105.

GOTHAN, W., 1906, Die fossilen Koniferenhölzer von Senftenberg. Abhandl. Preuss. Geol. Landesanst., N. F., Bd. 36, p. 164.

FUJIOKA, M., 1913, Studien über den anatomischen Bau des Holzes der japanischen Nadelbäume. Journ. of Coll. of Agric., Imp. Univ. Tokyo, Vol. 4, No. 4, p. 201.

IWAKI, T., 1918, Microscopical distinction of some Japanese coniferous woods. Bot. Mag. Tokyo, Vol. 32, pp. 178–189, 219–236.

JEFFREY, E. C., 1918, Anatomy of woody plants. Chicago.

KANEHIRA, R., 1926, Anatomical characters and identification of some important woods of the Japanese Empire. Dept. of For., Gov. Res. Inst. Taihoku, Formosa. Report No. 4.

CONWENTZ, H., 1882, Fossile Hölzer aus der Geologischen Landesanstalt. Jahrb. d. Preuss. Geol. Landesanst., Bd. 3, pp. 144–171.

KRÄUSEL, R., 1917, Die Pflanzen des schlesischen Tertiärs. Jahrb. d. Preuss. Geol. Landesanst., Bd. 38, Teil. 2, pp. 285–297.

KRÄUSEL, R., 1919, Nachträge zur Tertiärflora Schlesiens. Jahrb. d. Preuss. Geol. Landesanst., Bd. 40, pp. 418–452.

KRÄUSEL, R., 1919, Fossil-Koniferenhölzer. Paleontgraphica, Bd. 62, pp. 185–284.

OHARA, K., 1926, Zur Kenntnis fossiler Koniferenhölzer aus Japan. Japanese Journ. of Bot., Vol. 3, No. 2, pp. 97–109.

PENHALLOW, D. P., 1907, North American gymnosperms. Boston.

SEWARD, A, C., 1918, Fossil plants. Vol. 3. Cambridge.

YASUI, K., 1917, A fossil wood of *Sequoia* from the Tertiary of Japan. Ann. of Bot., Vol. 31, pp. 101–106.

YASUI, K., 1928, Studies on the structure of lignite, brown coal, and bituminous coal in Japan. Journ. of the Faculty of Science, Tokyo Imp. Univ., No. 70, Sect. 3, Vol. I, Part 4.
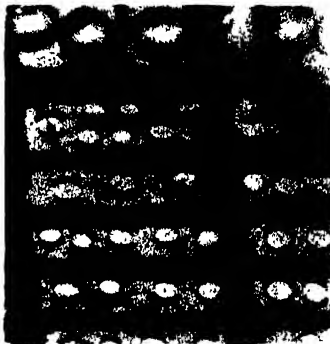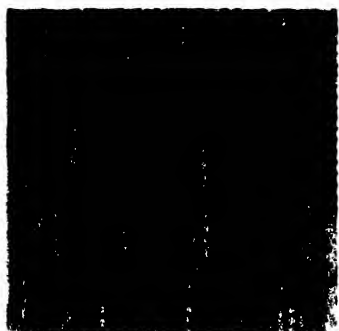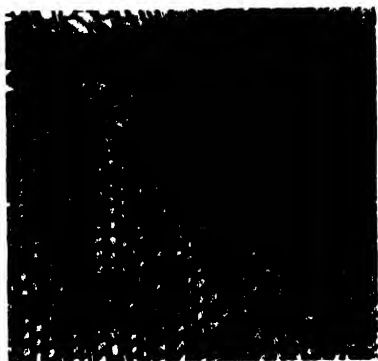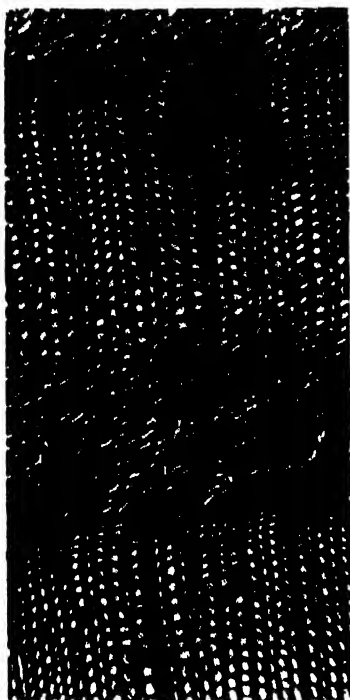
1

2

3

4

5

6

M. TAKAMATSU : Fossile Koniferenhölzer aus Sendai-Tertiär. I.

1

2

3

4

5

6

M. Takamatsu: Fossile Koniferenhölzer aus Sendai-Tertiär. I.

Cytological Studies on *Iris*.[1]

By

NATSU KAZAO.

(Biological Institute, Tôhoku Imp. University, Sendai)

In the genus *Iris* we have many species and varieties and some
of them are now commonly cultivated as ornamental plants. But
cytological studies on this genus are up to this time rather scanty.
Meiotic divisions in pollen mother cells were studied by STRASBURGER
(1900) in *I. germanica, I. Pseud-Acorus,* and *I. squalens* and by
MIYAKE (1905) in *I. florentina, I. pallida, I. spuria* and *I. Pseud-
Acorus.* By these investigations, it was shown that the haploid
chromosome number in the above mentioned species is twelve. Taking
the suggestion of Prof. TAHARA, the writer has for two years under-
taken cytological researches on the Japanese species of this genus.

The material was obtained mainly from the plants growing in the
garden of our Institute. The fixation of the pollen mother-cell was
made in FLEMMING's strong and weak solution, CARNOY's and BOUIN's;
among them the last mentioned solution gave the best results. In
the case of the root tip the material treated with FLEMMING's strong
solution was most satisfactory. Sections were cut 10–15 μ in thickness
and stained with HEIDENHAIN's iron alum haematoxylin. Up to the
present, six species have been investigated. They are as follows.

Names of plant	Haploid number of chromosomes	Diploid number of chromosomes
Iris Kaempferi, SIEB. var. *hortensis,* MAKINO	12	24
I. Kaempferi, SIEB. var. *spontanea,* MAKINO	12	24
I. sibirica, L var. *orientalis,* MAKINO	14	28

[1] A preliminary note of this investigation was already reported in Japanese last
year, in Bot. Mag. Tokyo, Vol. 42, with a brief English résumé.

I. laevigata, Fish. et Mey	16	32
I. florentina, L.[1]	—	48
I. gracilipes, A. Gray	18	36
I. japonica, Thumb.	—	54

As is shown in the above table, there are four kinds of haploid chromosome numbers in *Iris* namely 12, 14, 16 and 18. It is peculiar that these numbers make a regular arithmetical progression with the common difference 2. In the table haploid chromosome numbers of *I. florentina* and *I. japonica* are not given, because these two species do not perform regular meiotic divisions in their pollen mother-cells.

The heterotypic nuclear plates in the pollen mother-cells of *I. Kaempferi*, *I. sibirica*, *I. laevigata*, and *I. gracilipes* are shown in Text-fig. 1, a–d. In these plants the meiotic divisions in pollen mother-
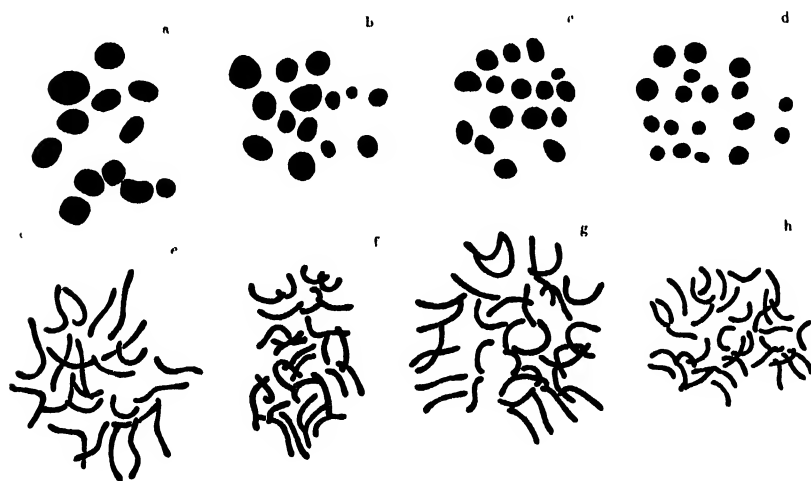
Text-fig. 1. Chromosomes of *Iris*. (× 1900)

a–d. Heterotypic nuclear division in pollen mother cells. e–h. Somatic nuclaer division. a, e. *I. kaempferi* var. *spontanea* (2 n – 24). b, f. *I. sibirica* var. *orientalis* (2 n – 28). c, g. *I. laevigata* (2 n = 32). d, h. *I. gracilipes* (2 n = 36).

cells proceed quite regularly, forming perfect-gemini as is usual in normal diploid plants. There are fairly significant differences in size

and shape among the gemini arranged in nuclear plate. The somatic nuclear plates of these four plants are also shown in Text-fig. 1, e–h.

As already mentioned, the meiotic divisions in the pollen mother cells of *I. florentina* and *I. japonica* are quite irregular. In prophase of the heterotypic divisions of these plants we can see distinctly a number of so-called trivalent chromosomes, connecting three homologous elements in different ways. The various shapes of these trivalent chromosomes are seen in Text-fig. 2 and 3: some being chains, others V- or Y-shaped, and still others like a ring with an attached rod. It should be noticed, however, that some chromosomes of these plants are connected together not in trivalent, but in bivalent chromosomes, thus leaving some in a univalent condition.

Text-fig. 2. *Iris florentina* (3 n = 18). (× 1900)
a. Late diakinesis, showing about 16 trivalent chromosomes. b. Side view of heterotypic metaphase in the pollen mother-cells. c. Polar view of the same. d. Somatic nuclear plate.

In anaphase of the heterotypic division, the three elements of these

trivalents are distributed unequally between the opposite two poles, consequently the pollen grains which are formed after the second division are irregular in size. Text-fig. 3, c shows the heterotypic

Text-fig. 3. *Iris japonica* (3 n = 54). (× 1900)

a. Polar view of the heterotypic metaphase of the pollen mother-cells. b. Side view of the same. c. Side view of the heterotypic anaphase. d. The homotypic anaphase. e. The diakinesis of megaspore mother cells. f. Somatic nuclear plate.

anaphase in pollen mother-cells of *I. japonica*, where can be seen about eight lagging chromosomes. d of the same figure shows the homotypic anaphase of the same.

The occurrence of trivalent chromosomes in heterotypic divisions has hitherto been reported in several autotriploid plants, namely in *Canna* (BELLING, 1921), *Datura* (BELLING and BLAKSLEE, 1922), *Tomato* (LESLLEY, 1925), *Primula* (IINUMA, 1926, ONO, 1927), *Prunus* (OKABE, 1927. 1928. DARLINGTON, 1928), etc. So it can be concluded that our two plants have also triploid chromosomes in their somatic cells. Somatic numbers of chromosomes of these two plants were determined in root-tips. It is 48 in *I. florentina* and 54 in *I. japonica*. The former plant is not native to our country but is commonly cultivated in our gardens. The latter species grows wild, however, throughout Japan. At present we can say nothing about the origin of this interesting plant. But *I. gracilipes*, also a native species in our land, has a certain resemblance to *I. japonica*. The haploid number of chromosomes of *I. gracilipes* is 18, that is exactly 1/3 of the somatic number of chromosomes of *I. japonica*. So it is very probable that the former species has some relation to the plant, from which the latter species originated. Compared to the chromosomes in somatic cells of *I. japonica*, those in *I. gracilipes* are much smaller in size, so it is doubtful that the former has a direct relation to the latter.

The examples of triploid plants have now become fairly numerous by the recent studies of several authors But most of these plants are garden varieties of wild species. The example of triploidy in distinct wild species, such as *I. japonica*, is still quite scanty. Recently NISHIYAMA has reported such a case in *Lycoris radiata*. It is an interesting fact that such species do not produce ripe fruits, propagation being performed only in vegetative ways.

Several authors have made a comparison between the size of cells of the triploid and diploid plants. In a similar way, measurments of the guard-cells of *I. japonica* and *I. gracilipes* were made. The results, average in 100 guard-cells, are shown in the following table.

Names of plant	Length (μ)	Width (μ)
I. gracilipes (2 n)	46.8	22.4
I. japonica (3 n)	29.1	13.0

In conclusion I wish to express my sincere gratitude to Prof. M. TAHARA for his kind suggestions and valuable criticisms throughout the course of this investigation.

_____ . _____   _____ . _____ , _____

POSTSCRIPT

After the completion of this manuscript, I have had the opportunity to see SIMONET's papar on *Iris*, " Le nombre des chromosomes dans le genre des *Iris*." The plants which he has investigated mostly differ from mine and only a few are the same. His results with the latter agree with mine quite well.

LITERATURE CITED.

1) BELLING, J. and BLAKESLEE, A. F. 1922. The assortment of chromosomes in triploid *Daturas*. Amer. Nat., Vol. 56, pp. 339-346.

2) BELLING, J. and BLAKESLEE, A. F. 1923. The reduction division in haploid, diploid, triploid and tetraploid *Daturas*. Proc. Nat. Ac. Sci. Washingston, Vol. 9, pp. 106-111.

3) BELLING, J. 1924. Homologous and similar chromosomes in diploid and triploid *Hyacinths*. Genetics, Vol. 10, pp. 59-71.

4) BELLING, J. 1927. The attachments of chromosomes at the reduction division in flowering plants. Journ. of Genetics, Vol. 18, pp. 177-205.

5) DARLINGTON, C. D. 1928. Studies in *Prunus*, I and II. Journ. of Genetics, Vol. 19, pp. 213-256.

6) IINUMA, M. 1926. Triploidy of chromosomes in garden varieties of *Primula Sieboldii*. Sci. Rep. of the Tôhoku Imp. Univ., 4th Series, Biology, Vol. 2, pp. 189-195.

7) JØRGENSEN, C. A. 1928. The experimental formation of heteroploid plants in the genus *Solanum*. Journ. of Genetics, Vol. 19, pp. 133-210.

8) KIHARA, H. and NISHIYAMA, I. 1928. New aspects of chromosome behaviour in pollen-mother-cells of tri-, tetra- and pentaploid wheat hybrids. Bot. Mag. Tokyo, Vol. 42, pp. 221-230.

9) LESLEY, M. M. 1926. Maturation in diploid and triploid Tomatoes. Genetics, Vol. 11, pp. 273-279.

10) MIYAKE, K. 1905. Über Reduktionsteilung in den Pollenmutterzellen einiger Monokotylen. Jahrb. Wiss. Bot., Bd. 42, pp. 83-120.

11) NISHIYAMA, I. 1928. Reduction division in *Lycoris*. Bot. Mag. Tokyo, Vol. 42, pp. 509-513.

12) OKABE. S. 1927. Cytological studies on *Prunus*. (A preliminary note). Bot. Mag. Tokyo, Vol. 41, pp. 398-404.

13) OKABE, S. 1928. Zur Cytologie der Gattung *Prunus*. Sci. Rep. of the Tôhoku Imp. Univ., 4th Series, Biology, Vol. 3, pp. 733-743.

14) ONO, T. 1927. Reducing division in triploid *Primula*. (A preliminary note). Bot. Mag. Tokyo, Vol. 41, pp. 601-604.

15) OSAWA, I. 1920 Cytological and experimental studies in *Morus* with special reference to triploid mutants. Bull. Seric. Exp. Stat. Japan, Vol. 1, pp. 318-369.

16) SIMONET, M. 1928. Le nombre des chromosomes dans le genre des *Iris*. C. R. Soc. Biol., Tome 99, pp. 1314-1316.

17) SIMONET, M. 1928. Le nombre des chromosomes chez les Iris des Jardins (*Iris germanica* Hort). C. R. Acad. Sci., Tome 187, pp. 840-841.

18) STRASBURGER, E. 1900. Histol. Beitr. Heft 6, pp 1-224.

19) TISCHLER, G. 1927. Pflanzliche Chromosomen-Zahlen. Tabulae Biologicae, Bd 4, pp. 1-83.

20) TOKUGAWA, Y. and KUWADA, Y. 1924. Cytological studies on some garden varieties of *Canna*. Japan. Journ. of Bot., Vol. 2, pp, 157-173.

Ovogenesis in *Coccophora Langsdorfii* (Turn.) Grev.

By

Masato Tahara

(Biological Institute, Tôhoku Imp. University, Sendai.)

As is well known, in Fucaceae the number of eggs developing in one oogonium is not constant; namely eight in *Fucus*, four in *Ascophyllum*, two in *Pelvetia*, and one in *Sargassum*. But in all these genera, no matter how many eggs come to function in one oogonium, three successive nuclear divisions always occur in each oogonium, and as a result eight nuclei are produced. In *Sargassum*, eggs newly discharged from the conceptacle have at first eight nuclei, which generally lie near the periphery of the egg. Therefore the three successive nuclear divisions in the oogonium can be in this case easily inferred.

In *Coccophora*, also a member of the family Fucaceae, however, the situation is totally different. In this alga the eggs which are developed also one in each oogonium have at the time of liberation from the conceptacle only one central nucleus.

Last year I made a preliminary investigation on the egg development of this alga (Tahara, 1928). Meiotic divisions in the oogonium and resulting four nuclei were observed without any difficulty. But the eight nucleate stage was not observed; and although it was only in one case, an egg was found which contained four nuclei, one large and three small, lying together in the central portion of the egg (Text-fig. 1). So I finally came to the conclusion that, " Four nuclei are produced as usual. However, it seems to me that three of these four nuclei
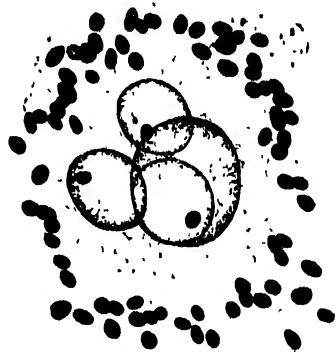
Text fig 1   Four nuclei grouped together in the central portion of an egg.   × 1200

are destined to degeration, and that the eight nucleate stage of the egg does not appear at all in this alga."

But more accurate knowledge relating to this point being quite desirable, this spring I went again to the Marine Biological Station at Asamushi to secure the material for further investigation. To my astonishment, an unexpected fact has been revealed by the observation on this new material, which will be described in the present paper. Also the main outline of the meiotic division in the oogonium of this plant will be treated at this opportunity, such studies being still quite scanty concerning this groups of marine algae.

The fixation of the material was carried out exclusively with FLEMMING's strong and weak solutions containing osmic acid with various modifications, among which the following one was proved to be generally useful for the fixation of the oogonium:

Stock solution of chromic acid (Sea Water 98 cc, saturated water
    solution of chromic acid 2 cc) . ... 70 cc
Sea Water ....30 cc
2% Osmic acid .... 5 cc
Glacial acetic acid .... 2.5 cc

The material remained in this mixture generally 6–8 hours. Fixation occured at various times of the day; the material was preserved at the sea shore for immediate use, fastened to a string, with a small stone for anchoring. Sections varying from $8\,\mu$ to $15\,\mu$ in thickness were stained, generally with HEIDENHAINs iron-alum haematoxylin.

The earliest developmental stage of the oogonium that I could find in the material of this year is figured in Pl. XXV. Fig. 1. This is perhaps an early stage of synapsis. The large nucleus situated in the center of the oogonium is seen surrounded closely by a multitude of chromatophores, except for a small clear space on one side of the nucleus. Against this clear space, is observed a small clump of chromatin-threads within the nucleus, arranged mainly in tiny loops protruding towards the center of the nucleus. Besides these threads there exist also in the interior of the nucleus irregular thread-like groupings of stainable substance, which are perhaps something other than the chromatin-granules. In the further stage of development, the loops of chromatin threads grow rapidly into the interior space of the nucleus, while on the other hand, the stainable substance

which hitherto existed throghout the nucleus disappears. (Pl. XXV, Fig. 2, 3).

In 1909 YAMANOUCHI published an accurate study on the mitosis of *Fucus*. His figure relating to the synapsis of this plant resembles mine quite closely. But according to his description, in a later stage of synapsis, the loops which have been so far connected together in a single continuous thread are segmented at the base and from a number of independent detached loops. He says, " The number of the loops is not easily counted from profile views ; however a section cut transversely through the loops showed that there are 61 cut ends of arms of the loops. Consequently, the number of loops is 32, each loop consisting of two arms." 32 being the haploid chromosome number of his material, the detached loops become thus in this case directly the bivalent chromosome, without passing through the spireme stage.

In my material, as is shown in Pl. XXV, Fig. 1, distinct a spireme stage comes after the synapsis. A continuous spireme-thread with strongly stainable knots here and there is seen running through the interior of the nucleus. The knots become more and more distinct and afterwards a stage that should be considered as diakinesis appears. The gemini, as the knots now can be called, instead of being distributed evenly in the periphery of the nucleus, as is usual in higher plants, are grouped together on one side of the nucleus. Their shapes are different, Y-, X-, O-, II- shapes being seen most frequently. The number of these gemini coincides with the haploid chromosome number of this plant : 32. In the culmination of this stage, the nucleolus disappears. Thus within the nucleus we can see only the gemini suspended in a faintly staining linin-network. As shown in Pl. XXV, Fig. 2, 3, 4, it is most frequently seen a small body, which is stained by haematoxylin like the nucleous and is found generally attached to the nucleolus. A similar body is described also in *Dictyota* (WILLIAMS, 1904), *Padina* (CARTER, 1927) and is called ' chromophilous spherule.'

At the complete formation of bivalent chromosomes the spindle-fibres begin to appear. To observe a definite process of spindle formation was, however, almost impossible in the case. Centrosomes which are recorded for the related genera, such as *Fucus* (YAMANOUCHI, 1909), *Sargassum* (TAHARA and SHIMOTOMAI 1925) and *Cystophyllum* (SHIMOTOMAI 1928) are not observed with certainty. This might be

perhaps attributed to the comparatively high systematic position of this alga.

In the metaphase, the bivalent chromosomes are arranged regularly in an equatorial plate, in the polar view of which 32 can be estimated as the haploid chromosome number of this plant (Pl. XXV, Fig. 13, 14).

After the completion of heterotypic mitosis, two fairly large inter-kinetic nuclei are formed (Pl. XXV, Fig. 10). The homotypic spindle that appears soon afterward is much smaller than the foregoing one (Pl. XXV, Fig. 12). Four nuclei naturally result from these nuclear divisions (Pl. XXV, Fig. 12). This was the last stage that I could find in the material obtained last year.

In this year's material, however, a further developmental stage was observed. Namley, the eight nucleate stage of the oogonium was observed. Thus the three successive nuclear division in the oogonium must be regarded as a normal course of development also in this plant. The case where only two successive nuclear divisions occur in the oogonium seems to appear only in the plants placed under abnormal conditions. Such is also the case in the species of *Sargassum*.
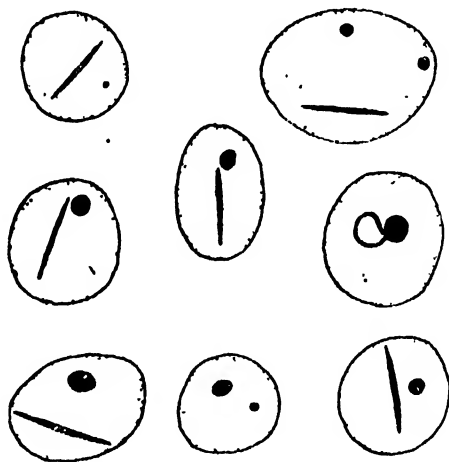
Text-fig 2.   Eight nuclei contained in a single oogonium.   ×2400

Text-Fig. 2 shows the eight nuclei contained in a single oogonium. One amongst them is much larger than the others and this is situated

at about the center of the oogonium, surrounded by a dense mass of cytoplasma, whilst the others lie near the periphery of the oogonium. It is a significant fact that each of these eight nuclei has a spindle-shaped body besides the nucleolus. This might be perhaps something other than the chromophilous body seen in the nuclei of heterotypic prophase (Text-fig. 2) (Pl. XXV. Fig. 13, 14). The 7 nuclei lying on the periphery of the oogonium disintegrate soon after their formation, and the oogonium comes to contain only a single nucleus in its central portion.

As already mentioned, the discharged eggs of *Sargassum* and also of *Cystophyllum* have eight nuclei distributed around the periphery of their body and none of these eight nuclei is specialized as a functional, central situated egg-nucleus. Thus on this point *Coccophora* decidedly differs from its related genera.

Coccophora is known as strictly endemic to the Japan Sea. It is the general opinion that the Japan Sea originated in a comparatively recent geological period, so the plants endemic to this sea could not be considered as the remnants of the primitive ones which have already disappeared in the Pacific ocean. On the contrary, they should be regarded as more highly specialized plants originating from their Pacific relatives. The structure of the eggs of *Coccophora Langsdorfii* appears to afford strong evidence for this opinion.

As for the segmentation mitosis which takes place after the fertilization it will here only be mentioned that about 60 chromosomes are seen arranged in a regular nuclear plate. Nor does the centrosome appear in this mitosis.

SUMMARY.

1. Three successive nuclear divisions occur in the oogonium of *Coccophora Langsdorfii.*

2. In the eight nucleate stage of the oogonium a single nucleus is situated in the center of the oogonium, whilst the other 7 are set aside to the periphery of the oogonium and are destined soon to disintegrate. The discharged eggs have only one central nucleus.

3. In the prophase of the heterotypic division in the oogonium typical synapsis-, spireme- and diakinesis-stage are observed.

4. The centrosome is not distinct in all nuclear divisions of this plant.

5. The chromosome number of this plant has been estimated to be 32 in the heterotypic and about 60 in the somatic divisions.

LITERATURE CITED

1) CARTER, P. W. 1927. The life-history of *Padina pavonia*. 1. The structure and cytology of the tetrasporangial plant. Ann. Bot., Vol. XLI, pp. 139–159.

2) KUNIEDA, H. 1928. On the development of the sexual organs and embryogeny in *Sargassum Horneri*, Ag. Jour. of Coll. of Agriculture, Imp. Univ. Tokyo, Vol. IX, pp. 383–396.

3) SHIMOTOMAI, N. 1928. Karyokinese im Oogonium von *Cystophyllum sisymbrioides*, T. Ag. Science Rep., Tôhoku Imp. Univ., 4th Ser., Vol. III, pp. 577–579.

4) TAHARA, M. and SHIMOTOMAI, N. 1926. Mitosen bei *Sargassum*. Science Rep., 4th Ser., Vol. I, pp, 189–192.

5) TAHARA, M. 1928. Contributions to morphology of *Coccophora Langsdorfii* (TURN.) GREV. Science Rep., 4th Ser., Vol. III, pp. 727–732.

6) WILLIAMS, T. LLOYD. 1404. Studies in Dictyotaceae. 1. The cytology of the tetrasporangium and the germinating tetraspore. Ann. Bot., Vol. XVIII, pp. 141–158.

7) YAMANOUCHI, S. 1909. Mitosis in *Fucus*. Bot. Gaz., Vol. XLVII, pp. 173–196.

EXPLANATION OF PLATE XXV.

Fig. 1–3. Synapsis.
Fig. 4. Spireme.
Fig 5–6. Diakinesis.
Fig. 7. Early metaphase.
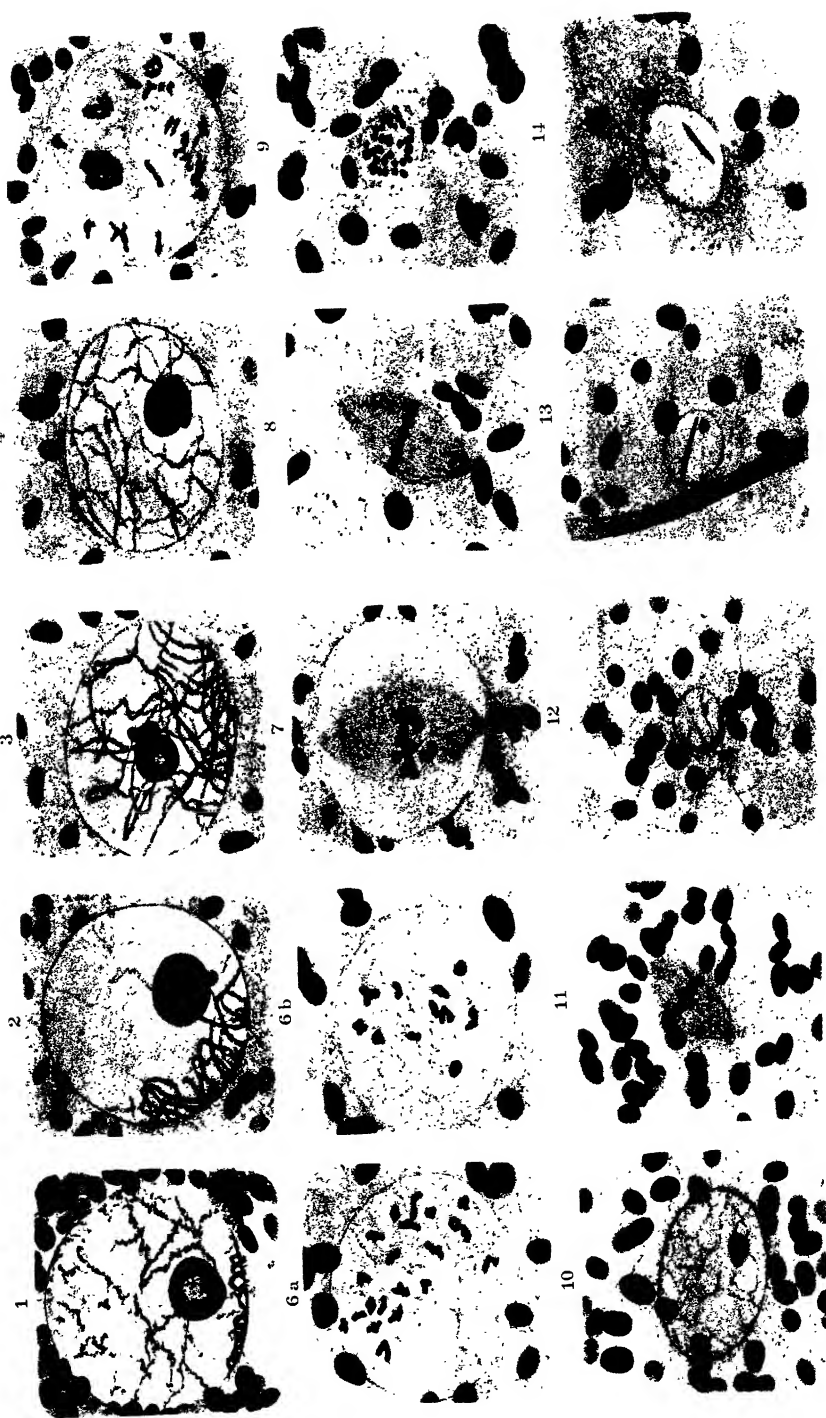Fig. 8–9. Heterotypic metaphase.
Fig. 10. Interkinese.
Fig. 11. Homotypic metaphase.
Fig. 12. Nucleus in four nucleate stage.
Fig 13. Eight nucleate stage. Nucleus figured is set aside to the periphery of the oogonium.
Fig. 14. The same stage. Central nucleus.

(All figures are magnified about 2200 times.)

M. Tahara: Ovogenesis in *Coccophora Langsdorfii* (Turn.) Grev.

Stimulating Action of Oxyphthalein[1] Colouring Matters on the Geotropism in Rice-Seedling with Special Reference to its Effect on the Growth in Length.

By

HATIWO TAKEDA.

(Biological Institute, Tôhoku Imperial University, Sendai.)

INTRODUCTION.

In 1925 POPOFF[2] showed that 0.01% eosin solution, treated 72 hours, hastens the growth of rice-seedling as much as 1.5 times that of the control, while in more concentrated solutions (to 0.1%, treated 24–72 hours) the inhibiting effect on growth is evident, causing the curved growth of its stem.

In the same year BOAS and MERKENSCHLAGER[2], and two years after BOAS[5] reported that the radicles of some seedlings treated with 0.1%–0.002% eosin solution in darkness, grow in all directions, resp. ageotropically, apparently quite independent of its poisoning and photodynamic action, while in 0.1% solution the upward growth of coleoptile is also disturbed.

Similar results are reported by v. D. MEER MOHR[4], who has found that both eosin and erythrosin inhibit the growth of many plants, so that they can not grow in a normal direction and become flowerless.

CLAUS[6] attributes the ageotropic growth of radicles caused by Eosin to the special character of this colouring matter which exhibits itself in strengthening the activity of diastase on the statolith-starch in perceptive regions.

In the present investigation dark-actions of Oxyphthalein colouring matters and their principal components on the positive or negative geotropism of radicle or plumule in rice-seedling were quantitatively studied, taking into consideration the influence on the growth in length caused by such chemicals; viz. Fluorescein, Eosin w. gelbl., Eosin bläul., two sorts of Erythrosin, Iodeosin, Eosin spritl., Cyanosin, Rose bengale, Gallein, resorcinol, o-phthalic acid, phthalic anhydride, bromine, iodine and chlorine.

METHOD.

As the material for this study, the seeds of pedigree No. 32 of Toyokuni, a race of *Oryza sativa* were used, which were harvested purely by the Agricultural Experiment Station of Miyagi Prefecture. Colouring matters used are of GRÜBLER's manufacture.

Fig. 1. Slightly germinated seeds of rice. ×4.

Equally and slightly germinated seeds as shown in Fig. 1 were selected as specimens and were treated with water solutions of colouring matters and chemicals above mentioned in definite concentrations at definite temperatures and for definite durations. Then they were washed with water, and sown in darkness on the germinator (Fig. 2) the bed of which just touches the surface of distilled water in the dish. This germinator was especially designed by the author to make free from disturbances in the growth of radicles and plumules.* Seed-

Fig. 2. The germinator. ×½.

*Over the brim of small PETRI-dish is stretched the clean cottonfibre-net which was soaked into the fused hard paraffin. This dish is then filled with distilled water and placed in a larger dish with a cover. The excess of water caused by the increase of temperature flow out from the inner into the outer PETRI-dish which serves as the moist chamber. The undulation of the surface of water which may happen at the removal of the germinator is checked by the net. On the bed of this germinator the poses of seedlings once gained are stably retained.

lings which are arranged on this germinator grow gradually and under circumstances thrust out radicles and plumules quite freely in every direction, receiving no restriction. Now the normal plumule ascends and the normal radicle descends vertically, while in abnormal cases seedlings put out plumules and radicles in various directions, so that they deviate from their normal position by certain angles, taking the straight, curved, waving, curling or twisted forms according to the sort of reagents used. After a suitable growth has taken place, the deviating angle of each plumule and radicle from the perpendicular at their base to the horizontal plane, that is, the angle between the line which connects the base and the point of the radicle or the plumule and the perpendicular is measured. In some cases to determine the percentage of abnormal specimens to the total the number of specimens which have radicles growing upwards over the horizontal plane is counted, and, if any, plumules growing horizontally or downwards under the horizontal plane are also counted. Then the seedlings are killed by 50% alcohol, and the length of radicles and plumules is measured.

In this way the results are obtained as they are given in the following chapters.

RESULTS.

I. THE ACTION OF OXYPHTHALEIN COLOURING MATTERS.

A. The influence of Eosin w. gelbl.. (Fig. 3)

Seedlings are treated with water solutions of Eosin w. gelbl. at 17°C - 22°C during 17 hours. Numericals indicate averaged values from 111 specimens each. The results are given in Table 1.

B. The influence of Eosin bläul.

Seedlings are treated with water solutions of Eosin bläul. at 28°C during 25 hours. Numericals are averaged values from 50 specimens each.

Eosin bläul. gives very similar figures of ageotropism to those of Eosin w. gelbl. (Fig. 3). The results are given in Table 2.

Fig. 3. Above: Specimens treated with 0.1% water solution of Eosin w. gelbl. at 15°C during 20 hours. Central one is a top view, while the others are lateral views. Below: Control. Lateral view. ×⅔. T. Soma phot.

TABLE 1.

Conc. of Eosin w. gelbl.	0.1%	0.01%	Control
Specimen which has radicle growing horizontally or upwards	87%	79%	0%
The form of plumule	horizontal	not erect	erect
Length of radicle	5.2 mm	9.9 mm	16.7 mm
Length of plumule	3.6 mm	4.5 mm	5.7 mm

TABLE 2.

Conc. of Eosin bläul.	0.1%	0.01%	0.001%	0.0001%	Control
Angle of deviation of radicle from its normal position	?	81°	29°	2°	1°
Angle of deviation of plumule from its normal position	84°	68°	37'	0°	0°
Length of radicle	0.4 mm	3.4 mm	7.0 mm	8.1 mm	8.1 mm
Length of plumule	2.4 mm	3.4 mm	5.1 mm	5.5 mm	5.5 mm

C. The influence of Erythrosin (α)*.

Seedlings are treated with 0.1% water solution of Erythrosin (α) at 21°C–24°C during 15 hours. Numericals are averaged values from 300 specimens each.

Erythrosin (α) gives very similar figures of ageotropism in radicle and plumule to those of Eosin w. gelbl. (Fig. 3) or Eosin bläul.

TABLE 3.

	0.1% Erythrosin (α)	Control
Specimen which has radicle growing horizontally or upwards	41%	0%
Specimen which has plumule growing horizontally or downwards	29%	0%
Length of radicle	3.6 mm	6.5 mm
Length of plumule	4.6 mm	7.0 mm

D. The influence of Erythrosin (β)*.

Seedlings are treated with saturated water solution of Erythrosin (β) at 22°C–23°C during 20 hours. Numericals are averaged values from 320 and 177 specimens respectively.

TABLE 4.

	Erythrosin (β)	Control
Specimen which has radicle growing horizontally or upwards	15%	0%
Specimen which has plumule growing horizontally or downwards	39%	0%
Length of radicle	4.9 mm	6.3 mm
Length of plumule	5.0 mm	5.5 mm

*There were two kinds of Erythrosin in GRÜBLER's manufactures in spite of their identical labels " Erythrosin puris." The one is very easily soluble in water and the solution is intensively fluorescent while the other is very slightly soluble in water and without fluorescence. For the sake of convenience the former is called in the present paper Erythrosin (α) and the latter Erythrosin (β). The chemical difference between them may probably lie in the point that Erythrosin (α) is tetrabromide and Erythrosin (β) biiodide.

It is worthy of notice that the solution of Erythrosin (β) which has no fluorescence, has a stronger effect on the negative geotropism of plumule than its action on the positive geotropism of radicle. In this respect the Erythrosin (β) is very similar to the case of Iodeosin which has fluorescence in solution, as one may see in the following lines.

E. The influence of Iodeosin.

Iodeosin is also very slightly soluble in water and fluorescent with moderate intensity. It has an effect especially on the negative geotropism of plumule. Fig. 4 shows such an example. This fact is of interest

Fig. 4. Lateral view of seedlings treated with 0.01% solution of iodeosin, as described in Table 6, showing the curious poses just as they stand on the bed of germinator. $\times \frac{3}{4}$.

if one takes into consideration the similar result with iodine (in KI) solution, as one may see in the next chapter.

TABLE 5.

Seedlings are treated with saturated water solution of Iodeosin at 21°C during 15 hours. Numericals are averaged values from 300 and 279 specimens respectively.

	Iodeosin	Control
Specimen which has radicle growing horizontally or upwards	7%	0%

	Iodeosin	Control
Specimen which has plumule growing horizontally or downwards	33%	0%
Length of radicle	4.9 mm	5.9 mm
Length of plumule	5.7 mm	5.2 mm

TABLE   6.

Seedlings are treated with 0.01% water solution of Iodeosin or Eosin w. gelbl. for comparison at 18°C–21°C during 24 hours. Numericals are averaged values from 50 specimens each.

	0.01% Iodeosin	0.01% Eosin w. gelbl.	Control
Angle of deviation of radicle from its normal position	30°	42°	10°
Angle of deviation of plumule from its normal position	84°	54'	3°
Length of radicle	11.9 mm	11.4 mm	16.9 mm
Length of plumule	9.1 mm	9.6 mm	10.1 mm

Iodeosin confuses the negative geotropism of plumule more intensively than the solution of Eosin w. gelbl. in the same concentration.

F.   The influence of Eosin spritl.

Eosin spritl. is also hardly soluble in water, but the solution is moderately fluorescent.   Its action is however negative, though it seems to have four atoms of bromine in the molecule, as is also the case with Eosin w. gelbl.

TABLE   7.

Seedlings are treated with saturated water solution of Eosin spritl. at 21°C during 15 hours. Numericals are averaged values from 300 specimens each.

	Eosin spritl.	Control
Specimen which has curled radicle	1%	0%

	Eosin spritl.	Control
Specimen which has non-erect plumule	3%	0%
Length of radicle	5.6 mm	5.9 mm
Length of plumule	4.8 mm	5.2 mm

TABLE 8.

Seedlings are treated with 0.01% water solution of Eosin spritl. or Eosin w. gelbl. for comparison at 21°C–18°C during 24 hours. Numericals are averaged values from 50 specimens each.

	0.01% Eosin spritl.	0.01% Eosin w. gelbl.	Control
Angle of deviation of radicle from its normal position	15°	42°	10°
Angle of deviation of plumule from its normal position	4°	54°	3°
Length of radicle	14.1 mm	11.4 mm	16.9 mm
Length of plumule	9.3 mm	9.6 mm	10.1 mm

The negative action in the case of Eosin spritl. is also evident, though the solution of Eosin w. gelbl. in the same concentration confuses the geotropism quite intensively.

G. The influence of Cyanosin.

The solution of Cyanosin has very faint fluorescence. Seedlings

TABLE 9.

	0.1% Cyanosin	Control
Specimen which has radicle growing horizontally or upwards	50%	0%
Specimen which has plumule growing horizontally or downwards	11%	0%
Length of radicle	5.6 mm	12.6 mm
Length of plumule	2.9 mm	6.0 mm

are treated with 0.1% water solution of Cyanosin at 25°C–23°C during
15 hours. Numericals are averaged values from 316 and 208 specimens
respectively. (Table 9).

H. The influence of Rose bengale.

Seedlings are treated with 0.1% water solution of Rose bengale
at 25°C–23°C during 15 hours. Numericals are averaged values from
208 to 332 specimens each.

TABLE 10.

	0.1% Rose bengale	Control
Specimen which has radicle grow-ing horizontally or upwards	9%	0%
Specimen which has plumule grow-ing horizontally or downwards	19%	0 ?
Specimen which has plumule twisted or waving, with point non-ascending	7%	0%
Length of radicle	6.2 mm	12.6 mm
Length of plumule	3.2 mm	6.0 mm

Rose bengale shows in solution no fluorescence. It confuses
especially the negative geotropism of plumule. But in more dilute
solution (0.01%) it can not cause such positive reaction, while Eosin
etc. in the same concentration more or less disturb the geotropism.

I. The influence of Gallein sicc. and Fluorescein.

These two colouring matters, particularly the latter are slightly
soluble in water, and in the solution Gallein shows no fluorescence
while Fluorescein exhibits strong fluorescence. Both of them inhibit
or accelerate the growth of radicle, but do not cause ageotropism at
all, as one may see from the following table. Seedlings are treated
with 0.004% water solution of Fluorescein or Eosin w. gelbl. for
comparison or with 0.01% water solution of Gallein sicc. at 21°C–18°C
during 24 hours. Numericals are averaged values from 50 specimens
each.

TABLE 11.

	0.004% Eosin w. gelbl.	0.004% Fluorescein	0.01% Gallein	Control
Angle of deviation of radicle from its normal position	49°	11°	12°	10°
Angle of deviation of plumule from its normal position	41°	5°	5°	3°
Length of radicle	12.4 mm	14.8 mm	23.4 mm	16.9 mm
Length of plumule	9.5 mm	11.0 mm	10.1 mm	10.1 mm

In the above table it is remarkable to note that 0.004% solution of Eosin w. gelbl. confuses geotropism moderately in opposition to the negative effect of Fluorescein in the solution of the same concentration.

J.   Comparison of actions of several Oxyphthalein colouring matters.

For the sake of comparison easily water-soluble colouring matters only are employed.   Among them the fluorescence of Eosin w. gelbl. is the most intensive, of Eosin bläul. and Erythrosin (α) the next and of Cyanosin the least in its intensity.   Rose bengale is not fluorescent at all.   Inhibiting action on growth and confusing action on geotropism seems also to be graded in the order of this intensity on the whole, so far as the researches are extended.

TABLE 12.

Seedlings are treated with 0.001 Mol water solution * of each colouring matter at 15°C–18°C during 20 hours.   Numericals are averaged values from 270 to 278 specimens each.

	Eosin w. gelbl.	Erythrosin (α)	Eosin bläul.	Cyanosin	Rose bengale	Control
Specimen which has radicle growing horizontally or upwards	71%	65%	45%	6%	0%	0%

* The Mol solutions are prepared after the formulae cited in " Farbstofftabellen ".[1]

	Eosin w. gelbl.	Erythrosin (α)	Eosin bläul.	Cyanosin	Rose bengale	Control
Specimen which has plumule growing horizontally or downwards	2% or more	4%	2%	0%	0%	0%
Specimen which has perfectly normally growing radicle and plumule	0%	6%	30%	85%	98%	100%
Length of radicle	8.9 mm	9.8 mm	12.6 mm	15.4 mm	15.3 mm	28.4 mm
Length of plumule	5.2 mm	4.7 mm	5.9 mm	5.8 mm	5.0 mm	7.7 mm

TABLE 13.

Seedlings are treated with 0.01% water solution of each colouring matter at 24°C-21°C during 46 hours. Numericals are averaged values from 50 specimens each.

	Eosin w. gelbl.	Eosin bläul.	Cyanosin	Rose bengale	Control
Angle of deviation of radicle from its normal position	75°	60°	30°	10°	6°
Angle of deviation of plumule from its normal position	63°	(5°	27'	6'	1°
Length of radicle	6.9 mm	7.6 mm	10.1 mm	10.6 mm	10.9 mm
Length of plumule	5.0 mm	5.2 mm	5.0 mm	4.6 mm	6.9 mm

It is conspicuous that Rose bengale inhibits especially the growth of plumule.

II. THE ACTION OF SEVERAL CHEMICAL COMPONENTS OF WHICH THE OXYPHTHALEIN COLOURING MATTERS CONSIST

According to SCHULTZ[1] almost all Oxyphthalein colouring matters are made from phthalic acid and resorcinol, and the differenciation among them is based upon the various combinations of these two nuclei with bromine, iodine, chlorine etc. respectively. So these essential components were also examined by means of the following

chemicals in respect to their influence on the geotropism and the growth of seedling.

A.   The influence of Resorcinol (GRÜBLER's).

Seedlings are treated with water solution of 1.0, 0.5, and 0.1% concentration at 18°C–20°C during 20 hours. Numericals are averaged values from 100 specimens each.

TABLE   14.

	Resorcinol			Control
	1.0%	0.5%	0.1%	
Specimen which has radicle growing horizontally or upwards	0%	0%	0%	0%
Specimen which has plumule growing horizontally or downwards	0%	0%	0%	0%
Specimen which has normal plumule but lacks radicle	78%	12%	0%	0%
Specimen which lacks both radicle and plumule	12%	0%	0%	0%
Specimen which has normal radicle and plumule	10%	88%	100%	100%
Length of radicle	0.1 mm	6.9 mm	15.1 mm	16.1 mm
Length of plumule	2.0 mm	4.8 mm	7.4 mm	7.9 mm

Resorcinol inhibits only the growth, but does not confuse the geotropism at all.

B.   The influence of o-phthalic acid and phthalic anhydride (KAHLBAUM).

These two chemicals confuse only the positive geotropism of radicle in a moderate intensity. Seedlings are treated with water solution of them at 30°C during 15 hours. Numericals are averaged values from 100 specimens each.

TABLE 15.

	o-Phthalic acid			Control
	0.5%	0.25%	0.125%	
Specimen which has radicle growing horizontally or upwards	0%	9%	12%	0%
Specimen which has plumule growing horizontally or downwards	0%	0%	0%	0%
Specimen which has normal plumule but lacks radicle	27%	74%	14%	0%
Specimen which lacks both radicle and plumule	73%	11%	0%	0%
Specimen which has normal radicle and plumule	0%	6%	74%	100%
Length of radicle	?	1.5 mm	17.0 mm	23.5 mm
Length of plumule	0.7 mm	5.5 mm	12.8 mm	16.6 mm

TABLE 16.

	Phthalic anhydride			Control
	0.5%	0.25%	0.125%	
Specimen which has radicle growing horizontally or upwards	0%	5%	11%	0%
Specimen which has plumule growing horizontally or downwards	0%	0%	0%	0%
Specimen which has normal plumule but lacks radicle	18%	76%	18%	0%
Specimen which lacks both radicle and plumule	82%	14%	0%	0%
Specimen which has normal radicle and plumule	0%	4%	71%	100%
Length of radicle	?	1.0 mm	17.8 mm	23.5 mm
Length of plumule	0.6 mm	4.8 mm	13.1 mm	16.6 mm

C.   The influence of Chlor-calc.

Seedlings are treated with saturated water solution of chlor-calc at 19°C during 20 hours.   Numericals are averaged values from 166 and 127 specimens respectively.

TABLE   17.

	Chlor-calc	Control
Specimen which has radicle grow-ing horizontally or upwards	4%	0%
Length of radicle	5.7 mm	12.9 mm
Length of plumule	5.1 mm	6.0 mm

Chlor-calc water which dissolves free chlorine in itself, also seems to act very slightly on the development of plumule.   Viz. it bends the points of plumule slightly in several specimens.   But it is doubtful whether this may be accounted for by the ageotropic growth caused by chlor-calc or chlorine.

The above result with chlor-calc may rather suggest that it is due to the character of chlorine itself.

D.   The influence of bromine. (Fig. 5).

Bromine acts chiefly on radicle to confuse its positive geotropism, but to some extent disturbs also the negative geotropism of plumule.
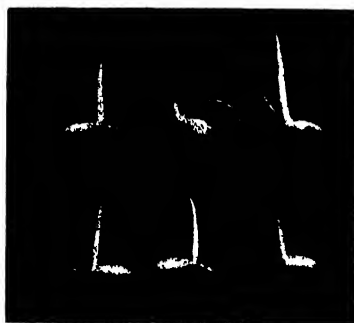

Fig. 5.   Ageotropism of radicles caused by 0.1%–0.05% bromine water; treated at ca. 20°C during 20 hours.   Lateral view.   Somewhat reduced.

This is worthy of notice, because the only difference between Eosin w. gelbl. and Fluorescein lie in the point whether it is a bromo-derivative or not.

TABLE   18.

Seedlings are treated with bromine water at 18°C–20°C during 20 hours. Numericals are averaged values from 100 specimens each.

Concentration at the beginning of treatment	Bromine		Control
	0.06%	0.03%	
Specimen which has radicle growing horizontally or upwards	34%	7%	0%
Specimen which has plumule growing horizontally or downwards	0%	0%	0%
Specimen which has normal radicle and plumule	46%	93%	100%
Length of radicle	3.4 mm	10.9 mm	16.1 mm
Length of plumule	5.0 mm	6.8 mm	7.9 mm

TABLE   19.

Seedlings are treated with bromine water at 20°C during 20 hours. Numericals are averaged values from 50 specimens each.

Concentration at the beginning of treatment	Bromine				Control
	0.2%	0.1%	0.05%	0.025%	
Angle of deviation of radicle from its normal position	Half of all specimens were killed.	64°	10°	16°	12°
Angle of deviation of plumule from its normal position	In the rest radicles did not appear,	6°	5°	4°	4°
Length of radicle	plumules grew somewhat horizontally	2.5 mm	8.6 mm	11.0 mm	14.9 mm
Length of plumule	and were very small	5.6 mm	9.0 mm	10.0 mm	9.3 mm

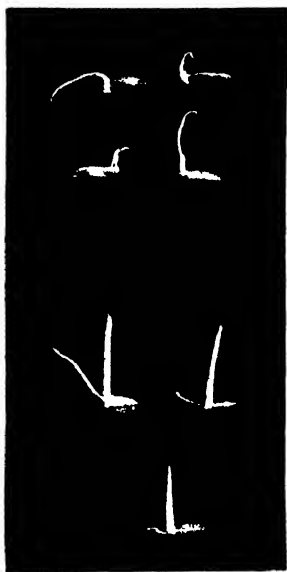### E. The influence of iodine. (Fig. 6).

Fig. 6   Above: The confusion of negative geotropism of plumules caused by 0.05% iodine dissolved in 0.1% KI water solution; treated at ca. 19°C during 21 hours. Below: Specimens which were treated with 0.03% iodine dissolved in 0.04% KI water solution at 30°C during 17 hours. Lateral views. Somewhat reduced.

Iodine is hardly soluble in water; so it is necessary to take iodine dissolved in KI solution as a stimulant, which is intensive enough in effect for this experiment.

Dilute solution of iodine accelerates the growth only. A little more concentrated solution inhibits the growth and confuses the positive geotropism of radicle. A rather concentrated solution in which radicles are no longer allowed to grow further confuses strongly the negative geotropism of plumules, as shown in Fig. 6. This latter fact coincides with that in case of Iodeosin where the negative geotropism of plumule is also disturbed. The only difference between Iodeosin and Fluorescein is whether it is a iodo-derivative or not.

When the seedlings are treated with 0.05% iodine dissolved in 0.1% water solution of KI at 18°C–19°C during 21 hours, the negative geotropism of plumules is strongly confused, though they become somewhat slender, apparently owing to the poisoning action of iodine, showing many curious figures of downward growth of plumules, as they are reproduced in Fig. 6.

TABLE   20.

Seedlings are treated with saturated water solution of iodine at 21°C–24°C during 15 hours.   Numericals are averaged values from 98 specimens each.

	Iodine water	Control
Specimen which has radicle growing horizontally or upwards	4%	0%

	Iodine water	Control
Specimen which has plumule growing upwards but shows crooked or descended point	20%	0%
Length of radicle	8.6 mm	6.5 mm
Length of plumule	5.9 mm	7.0 mm

N. B. Almost all plumules of treated specimens are thick, wide and obtuse-pointed.

TABLE 21.

Seedlings are treated with 0.1% water solutions of NaI and KI at 32°C during 20 hours. Numericals are averaged values from 100 to 110 specimens each.

	NaI	KI	Control
Specimen which has radicle growing horizontally or upwards	0%	1% or less	0%
Specimen which has plumule growing horizontally or downwards	0%	0%	0%
Length of radicle	15.0 mm	14.6 mm	13.3 mm
Length of plumule	10.7 mm	11.3 mm	11.0 mm

TABLE 22.

Seedlings are treated with iodine (in KI) solutions at 30°C during 15 hours. The solvent is less concentrated than 0.1% as to KI. Numericals are averaged values from 100 specimens each.

Concentration at the beginning of treatment	Iodine			Control
	0.08%	0.06%	0.04%	
Specimen which has radicle growing horizontally or upwards	0%	indistinct, because of smallness of radicles	60%	0%
Specimen which has descended, horizontal or twisted plumule	0%	indistinct, because of smallness of plumules	12% or more	0%

Concentration at the beginning of treatment	Iodine			Control
	0.08%	0.06%	0.04%	
Specimen which has been killed	100%	24%	4%	0%
Length of radicle	0.0 mm	0.1 mm	4.6 mm	20.1 mm
Length of plumule	0.2 mm	0.7 mm	2.5 mm	10.2 mm

TABLE 23.

The influence of iodine (in KI) solution and the solvent itself. Seedlings are treated at 30°C during 17 hours. Numericals are averaged values from 50 specimens each.

Concentration at the beginning of treatment	0.03% Iodine in 0.04%KI	0.04% KI	Control
Angle of deviation of radicle from its normal position	47°	20°	18°
Plumule	erect but obtuse-pointed	normal	normal
Length of radicle	8.2 mm	17.4 mm	14.1 mm
Length of plumule	10.0 mm	15.1 mm	13.9 mm

CONCLUSION.

If the molecular formulae of colouring matters used may be assumed to comply with those of dyes which are described in "SCHULTZ: Farbstofftabellen"[1] in the coincident names respectively, the meaning of the above results may probably be explained as follows.

Free phthalic acid confuses geotropism. But Fluorescein and Gallein have no such action. In these dyes phthalic acid is restricted to the resorcinol-nucleus. And the thus restricted phthalic acid-nucleus seems to have no more such ageotropic action. From this consideration it is presumed that the ageotropism caused by Oxyphthalein colouring matters is independent of their phthalic acid-nuclei.

The mere addition of tetra bromine to Fluorescein causes the ageotropism above mentioned. And free bromine has also such action. Furthermore, this tetrabromide of Fluorescein, i. e. Eosin w. gelbl.

confuses more intensively the geotropism than Eosin bläul. which is the dibromide of the same. Therefore it is probable that the existence of bromine may be part of the cause of such ageotropism.

Bromine resp. bromides among Oxyphthalein colouring matters act chiefly to disturb the positive geotropism of radicle, and in a subordinated degree to confuse the negative geotropism of plumule also.

On the other hand Iodeosin, Erythrosin (β) and Rose bengale are iodides of Fluorescein and confuse mainly the negative geotropism of plumule. Free iodine, accompanying some poisoning actions, causes the downward curvature of the plumule. Iodine resp. iodides among Oxyphthalein colouring matters also disturb to some extent the positive geotropism of radicle. Then the existence of iodine may be an another cause of such ageotropism, especially of the plumule. Iodine or bromine combines with the resorcinol-nucleus of each colouring matter.

The resorcinol-nucleus, which constitutes each Oxyphthalein colouring matter together with phthalic acid-nucleus, is probably independent of the ageotropism caused by Oxyphthalein colouring matters.

Now Eosin w. gelbl., Erythrosin (a) and Cyanosin are all tetra-bromides. But the ageotropic action of Eosin w. gelbl. is the strongest, while Erythrosin (a) is the next, and Cyanosin is the weakest of all. On the other hand chlorine is combined with the phthalic acid-nucleus in each of the last two dyes, viz. Erythrosin (a) is bichloride and Cyanosin tetrachloride. Then chlorine seems to act antagonistically to bromine in respect to the confusing action on the geotropism. Similarly chlorine is also antagonistic to iodine. Namely Iodeosin, which is a tetraiodide, and has no chlorine in the molecule confuses the geotropism more strongly than Rose bengale, which is a tetraiodide, and simultaneously bichloride.

Eosin spritl. has no action of disturbing geotropism in spite of the tetrabromide in its chemical structure. Perhaps this is due to the existence of methyl- or ethyl-group which combines with the phthalic acid-nucleus of this dye.

SUMMARY.

Among Oxyphthalein colouring matters, bromides or iodides confuse geotropism in rice-seedling by dark-action.

Roughly speaking, such action is in proportion to the intensity of fluorescence produced by the water solution of each dye in light.

Dyes which are iodides disturb mainly the negative geotropism of plumule, while dyes which are bromides disturb the positive geotropism of radicle on the whole.

It is probable that the cause of ageotropism which results from the treatment with such colouring matters is based upon the existence of iodine or bromine which combines with the resorcinol-nucleus of these dyes.

This work has been directed by Prof. Dr. Y. YAMAGUTI, to whom I here wish to express my gratitude.

LITERATURE CITED.

1) SCHULTZ, G., 1920: Farbstofftabellen. 5. Aufl., pp. 196–204.

2) POPOFF, M., 1925: Zellstimulantien und ihre theoretische Begründung. Zellstim.-Forsch., Bd. 1, Heft 1, pp. 27–28.

3) BOAS, F. und MERKENSCHLAGER, F., 1925: Reizverlust, hervorgerufen durch Eosin. Ber. d. D. Bot. Ges., Bd. 43, pp. 381–390.

4) V. D. MEER MOHR, J. C., 1926: Über die Wirkung von Eosin, Erythrosin- und Methylenblau-Lösungen auf Keimung und Wachstum einiger Pflanzen. Rec. d. trav. bot. néerl., Vol. 23, pp. 245–262.

5) BOAS, F., 1927: Zur Kenntnis der Eosinwirkung auf das Wachstum der Wurzeln. Ber. d. D. Bot. Ges., Bd. 45, pp. 61–64.

6) CLAUS, G., 1929: Über die Dunkelwirkung fluoresziereder Farbstoffe auf Diastase. Biochem. Z., Bd. 204, pp. 456–466.

On the Inheritance of Some Characters in *Glycine Soja*, Bentham (Soy-Bean).

By

Fumi Takagi.

(Biological Institute, Tôhoku Imp. University, Sendai)

(With Plate XXVI)

The inheritance of various characters in the soy-bean has been already studied by several authors. Namely Terao (1918) studied a maternal inheritance in cotyledon colours; Takahashi and Fukuyama (1919) studied the inheritance of the characters relating to cotyledon, seed-coat, hilum, leaf and others; Woodworth (1921) made an investigation on the inheritance of the colours of several parts of the plant, that is of cotyledon, seed-coat, hilum, and pubescence; Nagai reported several times on the inheritance of the soy-bean, in the latest report (1926), he published, amongst others, the results of the investigation on the stem and leaf form, the seed size, the seed-coat and flower colours.

"Kurakake", one form of the soy-bean, which has a saddle-like pattern on the seed-coat, is fairly common in Sendai. Another from of the soy-bean, which is called "Keitômame" or "Shakujômame" (*Glycine Soja*, Bentham mut. *fasciata* Takahashi) has a fasciated stem and is sometimes cultivated in the same district.

Professor Tahara planned to investigate the inheritance of these two plants. The cross was made for the first time in the spring of 1924, but no seed was produced. He made another attempt in 1925, and obtained some seeds from the cross "Keitômame" (-♀) × "Kurakake" (♂). In 1926, 2 F_1 plants grew from those seeds, and thereafter he transferred this work to the writer, who was hoping to study something in this direction. The writer continued the investigation in two successive generations. Some results will here be briefly reported. At the outset, it will be purposeful to enumerate the characters of the two plants.

I. THE CHARACTERS OF BOTH PARENT PLANTS.

The characters of both plants are shown in the following table.

TABLE 1.

	" Keitômame "	" Kurakake "
Stem form	fasciated	normal
Leaf colour	green	green
Flower colour	white	purple
Size of seed	small and round	large and flat
Hilum colour	brown	black
Pattern	no pattern	saddle (colour black)

The stem of " Keitômame " is normal when young, but fasciates gradually as it grows. In maturity, the stem form is quite different from that of " Kurakake ". The fasciated stem has only a few small branches (Pl. XXVI, Fig. 1). The flowers are crowded at the end of the stem, but are also seen scattered on other parts of the plants. With regard to the morphology of " Keitômame ", TAKAHASHI and FUKUYAMA (1919) have already published a report.

II FASCIATION.

The F_1 plants arising from the cross " Keitômame " $\times$ " Kurakake " have a non-fasciated stem. The segregation in F_2 occurred in a monohybrid ratio (Table 2).

TABLE 2.

	Normal	Fasciated
Observed	118	41
Expected (3:1)	119.25	39.75
Deviation	−1.25	+1.25
Mean error	5.46	5.46

In F_3 generation, the progenies of F_2 plants segregated in three types; the first bred true for the non-fasciated; the second bred

true for the fasciated, the third segregated in the non-fasciated and fasciated in the ratio 3:1. The following table refers only to those individuals of the last mentioned type.

TABLE 3.

Progenies of the third type of F_2 plants.

Pedigree number of F_2 plants	Normal	Fasciated
4	31	10(4)
13	44(1)	10(3)
33	23	12(4)
36	32(1)	17(7)
40	41	9(4)
42	24	8
64	48(3)	6(4)
65	42(1)	13(8)
82	31	6(2)
86	33	10(5)
93	27	14(8)
109	27	12(9)
111	47	20(13)
112	27	9(2)
113	36	14(5)
Total	513	170
Expected (3:1)	512.25	170.75
Deviation	+0.75	-0.75
Mean error	11.32	11.32

The above result coincides with that of NAGAI (1926) who made also a similar experiment.

In passing, it may be worth mention here that the flowers on the individuals with fasciated stems have sometimes 2 or 3 pistils. In the above table the numbers in the parenthesis show the numbers of the plants which produced two or more pods in the same flower. Ten fasciated plants of F_3 generation were chosen for an accurate observation, and ten flowers on each individuals were examined as to the number of pistils. The results are given in Table 4.

When a flower has two or three pistils, we can naturally expect the production of two or three pods in the same flower. But such a case does not often occur. If the two pods develop in the same

TABLE 4.

Number of pistils in flower	1	2	3
Plant number			
1	3	7	0
2	1	9	0
3	7	3	0
4	8	2	0
5	7	3	0
6	6	3	1
7	7	3	0
8	4	6	0
9	7	3	0
10	5	5	0
Total	55	44	1

flower, generally they are of unequal size, one becoming sometimes only a rudiment (Pl. XXVI, Fig. 3, Text-fig. 1). Also, fairly often
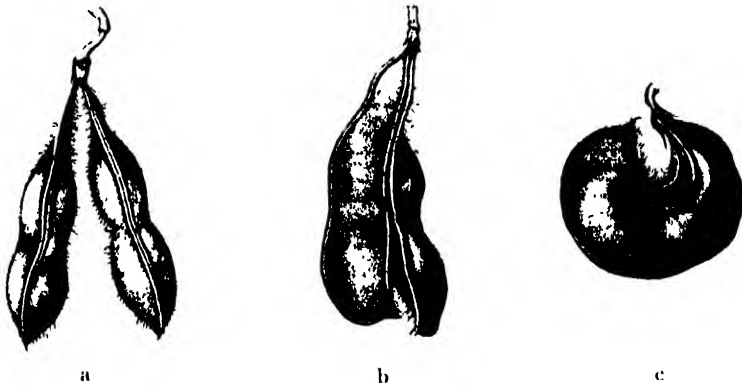
Text-fig. 1. Pods produced in flowers which have two or more pistils. a. Two distinct pods of equal size produced in one flower. b. Two pods produced in one flower partly fused. c. Three pods developed in one flower united together into a single body. The two of the three pods become, however, only rudimentary. ×1.

two pods developing in the same flower fuse together into a single body of prismatic shape, triangular in cross section, at maturity splitting in three valves, the inner valves of the two pods being united to a single valve. The suture line in this coalescence is only faintly visible in a ripened fruit and does not split even in maturity (Text-fig. 2).

Pods of such a nature have generally two malformed seeds of unequal size in each placenta, which are exclusively seen on the line formed by the union of two outer valves of the two pods.

III. CHLOROSIS.

In this crossing, the parents have green leaves and the F_1 plants are the same as the parents. But in the F_2, segregation occurred in the ratio, the normal type with green leaves 15 and the chlorosis type with greenish yellow leaves 1.

Text-fig. 2. Dehiscence of a pod which has developed from the entire fusion of two pods. The pod splits into three valves. × 1.

TABLE 5.

	Green	Greenish yellow
Observed	152	7
Expected (15:1)	149.06	9.94
Deviation	2.94	2.94
Mean error	3.05	3.05

The plants of the chlorosis type, when young, look like the normal ones, but in later development their leaves gradually turn greenish yellow and the plants becomes stunted in appearance. Accordingly, only very small and faintly coloured seeds are produced in these plants. The ratio 15 : 1 obtained in F_2 generation suggests two complementary factors, as **A** and **B**. From this assumption it follows that in the presence of either one or both of these factors, the leaves become green, but in the absence of both, the leaves become greenish yellow. Thus in genetic composition the parents of this cross are **AAbb** and **aaBB** respectively, and F_1 is **AaBb**. In the F_2 generation, one part out of 16 is **aabb** in composition and has greenish yellow leaves. The plants with greenish yellow leaves bred true for this character in the F_3 generation. The F_2 plants bearing green leaves bred differently in F_3 generation, as was expected; 7 parts out of 15 bred

true in F_3, 4 produced green and greenish yellow in an approximate $3:1$ ratio; and the remaining 4 out of 15 behaved like the F_1 plants, giving again an approximate $15:1$ ratio. The data are presented in Table 6.

TABLE 6.

Progenies from F_2 individuals with greenish yellow leaves.

Pedigree number of F_2 plants	Green	Greenish yellow
165	0	36

Progenies of F_2 plants bearing green leaves.

Pedigree number of F_2 plants	Green	Greenish yellow
4	42	0
13	51	0
28	51	0
82	37	0
86	43	0
88	41	0
90	73	0
91	45	0
93	41	0
98	74	0
99	47	0
108	36	0
112	37	0
Total	618	0
33	27	10
36	42	7
40	37	10
42	31	6
65	45	10
67	44	12
92	39	12
95	51	17
100	29	7
106	25	6
111	55	12
Total	425	109
Expected (3:1)	400.5	133.5
Deviation	24.5	24.5
Mean error	10.01	10.01

Pedigree number of F₂ plants	Green	Greenish yellow
27	36	4
31	47	4
64	54	1
85	41	2
87	43	4
89	29	2
107	83	6
109	37	2
110	23	3
113	48	2
152	34	4
Total	485	34
Expected (15:1)	486.56	32.44
Deviation	−1.56	+1.56
Mean error	5.51	5.51

IV. FLOWER COLOUR.

As for flower colour, a monohybrid ratio was obtained in F_2 and F_3 generation. Thus my results entirely coincide with NAGAI's. Data are shown in Table 7.

TABLE 7.

Flower colour in F_2 generation.

	Purple	White
Observed	122	37
Expected (3·1)	119.25	39.75
Deviation	+2.75	−2.75
Mean error	5.46	5.46

Flower colour in F_3 generation.

	Purple	White
Observed	500	152
Expected (3:1)	489	163
Deviation	+11	−11
Mean error	11.06	11.06

V. SIZE OF SEEDS.

The seeds of "Keitômame" are small and those of "Kurakake" are large, and the size of the seeds of F_1 plants are about intermediate between the size of the parent seeds. The data of the parents and F_1 are as follows.

TABLE 8.

	P ("Keitô")	P ("Kura")	F_1
Mean value	129.86 mg.	361.51 mg.	237.35 mg.
Number of seeds used for measurement	181	179	651
Standard deviation	25.63 mg	45.58 mg	39.47 mg.
Mean error of mean value	0.14 mg.	0.25 mg	0.06 mg.

Segregation in F_2 and F_3 is very complex, and no description is given here.

VI. HILUM AND SADDLE PATTERN COLOUR.

Both parent plants differ in hilum colour. In the seeds of "Keitômame" it is brown, while in the seeds of "Kurakake" it is black. The F_1 plants resulting from the cross between these parents bore seeds with the black hilum like that in the seeds of "Kurakake". In the F_2 generation, segregation occurred in the ratio, 3 black and 1 brown. The data are given in Table 9.

TABLE 9.

	Black	Brown
Observed	122	37
Expected (3:1)	119.25	39.75
Deviation	+2.75	- 2.75
Mean error	5.46	5.46

WOODWORTH reports a datum concerning the hilum colour inheritance. He says " Black hilum is dominant to brown hilum, and in F_2 a ratio of 9 black to 7 brown was obtained. This indicates that there are two factors, complements of each other, which are necessary to produce black hilum. In the absence of either one or both, the hilum is brown." Owing to the difference of the materials, he has obtained a different segregation in F_2 generation.

The seed of " Kurakake " has a saddle pattern around the hilum and " Keitômame " has no saddle. In F_1 generation, only a faintly coloured portion is discernible around the hilum (Pl. XXVI, Fig. 2). Segregation occurred in monohybrid ratio in F_2 generation. The data are shown in Table 10.

TABLE 10.

	No saddle	Saddle
Observed	111	48
Expected (3·1)	119.25	39.75
Deviation	8.25	8.25
Mean error	5.46	5.46

Namely, the saddle pattern in soy-bean is recessive to the non-saddle pattern. The pattern colour is always the same as the hilum colour. So it appears that an allelomorphic pair **Rr** is concerned with the colour of the both parts of the seed. Both parts become black in colour with the existence of **R,** and become brown in the absence of **R.** To explain the saddle pattern, it is necessary to assume an inhibiting factor **K.** Thus " Kurakake " has the composition **RRkk** and " Keitômame " **rrKK,** and F_1 **RrKk.** In F_2 generation, dihybrid segregation in the ratio $9:3:3:1$ was seen. The data are shown in Table 11.

In the F_3 generation, the plants with brown hilum and brown saddle bred true for these characters. The other F_2 plants bred differently. Some examples are shown in Table 12.

TABLE 11.

	Black hilum, non saddled	Black hilum, saddled	Brown hilum, non saddled	Brown hilum, saddled
Observed	82	40	29	8
Expected (9:3:3:1)	89.4	29.8	29.8	9.9
Deviation	−7.4	+10.2	−0.8	−1.9
Mean error	6.25	4.92	4.92	3.05

TABLE 12.

A. Progenies of F_2 plants bearing seeds non-saddled, with brown hilum.

Pedigree number of plants	Brown hilum, non-saddled	Brown hilum, saddled
64 (rrkk)	54	0
65⎰ (rrKk) 67⎱	39 49	14 17
Total	88	31
Expected (3:1)	89.25	29.75
Deviation	−1.25	+1.25
Mean error	4.72	4.72

B. Progenies of F_2 plants bearing seeds with black saddle.

Pedigree number of plants	Black hilum, saddled	Brown hilum, saddled
4⎫ 13⎪ 27⎪ 28⎬ (Rrkk) 31⎪ 33⎪ 36⎪ 40⎭	25 33 24 40 36 27 22 32	11 16 9 11 13 6 8 12
Total	239	86
Expected (3:1)	243.75	81.25
Deviation	−4.75	+4.75
Mean error	7.81	7.81

C   Progenies of F$_2$ plants bearing seeds non-saddled, with black hilum.

Pedigree number of plants	Black hilum, non-saddled	Black hilum, saddled	Brown hilum, non-saddled	Brown hilum, saddled
109 110 111 112 (RRKK)	39 34 57 35	0 0 0 0	0 0 0 0	0 0 0 0
Total	165			
86 89 92 100 107 (RRKk)	20 27 29 31 64	14 9 16 5 20	0 0 0 0 0	0 0 0 0 0
Total	180	64		
Expected (3 1)	183	61		
Deviation	3	+3		
Mean error	6.76	6.76		
85 90 93 106 (RrKK)	93 53 25 21	0 0 0 0	14 16 15 9	0 0 0 0
Total	130		54	
Expected (3 1)	138		46	
Deviation	8		+8	
Mean error	5.88		5.88	
82 87 86 91 95 98 99 108 113 152 (RrKk)	22 24 30 26 36 40 31 22 24 20	7 12 8 6 12 10 9 7 9 8	6 7 9 8 15 14 5 4 12 5	3 2 4 4 5 2 2 3 5 4
Total	275	88	85	34
Expected (9:3:3:1)	271.125	90.375	90.375	30.125
Deviation	+3.875	2.375	5.375	+3.875
Mean error	10.89	8.57	8.57	5.31

As a matter of course we can distinguish two kinds F_2 individuals bearing the seeds with black saddle. In genetic composition the one is **Rrkk** and the other is **RRkk**. **Rrkk** individuals can sometimes be detected at a glance from the **RRkk,** because the seeds of those plants **(Rrkk)** have often brown flecks scattered through the black area. In my reserch only the individuals with such a peculiarity were chosen for the examination of F_3 generation. Table 12, B does not contain, as a consequence, any data concerning the plants of the other type **(RRkk).**

SUMMARY.

1) The fasciation of the stem is caused by single genetic factor which is recessive to the factor for non-fasciation. Some flowers on the fasciated individuals have two or more pistils. The pods produced on such a flower differ considerably from the normal ones.

2) Parents and F_1 plants have normal green leaves. But concerning this point segregation occurs in F_2 in the ratio, 15 green : 1 chlorosis. This result was ascertained in the examination of F_3 generation.

3) The purple flower colour is dominant to the white.

4) The seeds of "Keitômame" have the brown hilum and no saddle pattern, and those of "Kurakake" have the black hilum and black saddle pattern. The seeds of F_1 plants arising from the cross between these two have the black hilum and no saddle pattern. In the F_2 generation the dihybrid ratio $9:3:3:1$ was observed.

LITERATURE CITED.

1) NAGAI, I. 1921. A genetico-physiological study on the formation of anthocyanin and brown pigments in plants. Journ. Coll. Agric. Imp. Univ. Tokyo, Vol. 8, No. 1, pp. 1-92.

2) NAGAI, I. and SAITO, S. 1923. Linkage factors in soy-bean Jap. Journ Bot, Vol. 1, No. 3, pp. 121-136.

3) NAGAI, I. 1926. On the inheritance of soy-bean (In Japanese). "Nogyo ovobi Engei", Vol. 1, No. 1, pp. 4-14. Vol. 1, No. 2, pp. 107-118.

4) TAKAHASHI, Y. and FUKUYAMA, J. 1919. Morphological and genetic studies on the soy-bean (In Japanese). Hokkaido Agric Exp St., Rep No. 10 pp 1-100.

5) TERAO, H. 1918. Maternal inheritance in the soy-bean. Americ Nat, Vol 52, pp. 51-56.

6) WOODWORTH, C. M. 1921. Inheritance of cotyledon, seed-coat, hilum and pubescence colours in soy-bean. Genetics, Vol. 6, pp 487-553.

EXPLANATION OF PLATE.

Fig. 1. The both parents. ×1/5.

Eig. 2. The two left in above line, "Keitômame". The other two in the same line, "Kurakake". The two below F₁. ×1.

Fig. 3. Anomalous pods produced in flowers which have two pistils   1
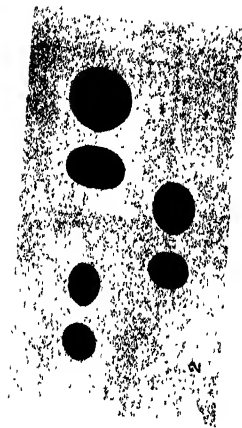
F. TAKAGI: Inheritance in *Glycine Soja*.

Rhizoidenentwicklung im Embryo von *Cystophyllum*.

VON

SAKUICHI OKABE.

(Biologisches Institut der Kaiserlichen Tôhoku Universität, Sendai).

Die letzten Untersuchungen von TAHARA über die Rhizoidenbildung bei der Embryogenese verschiedener Arten von Fucaceen haben neue, interessante Resultate gebracht. Betreffs der Rhizoidenbildung bei der Embryogenese von *Cystophyllum sisymbrioides* publizierte er schon vor sechzehn Jahren eine kurze Mitteilung. Dort schreibt er: „The segmentation process in the rhizoid cell of this plant differs considerably from that above described; that is, in this plant before the rhizoid formation begins, the rhizoid cell is already divided into about 30 small cells. Text-fig. 3 is the surface view of the rhizoidal portion of the sporeling and text-fig. 4 presents a median longitudinal section of the same. In the figure, we can see the two-storied arrangement, a state which is never found in *Sargassum*."

In diesem Frühling begab ich mich, auf Anraten von Herrn Prof. Dr. M. TAHARA, nach Misaki zur Biologischen Station der Kaiserlichen Tokyo-Universität und beobachtete eingehend die Entwicklung der Rhizoiden dieser Alge. Am 17. März 1929 konnte ich an der Küste von Misaki zum ersten Mal die Oogonien von *Cystophyllum sisymbrioides* sich sehr üppig entleeren sehen. Dieses Material diente zu meiner Untersuchung.

Wie TAHARA schon bemerkte, sind simultan entleerte Oogonien von unzähligen, sich verwirrender Paraphysen an den Rezeptakeln befestigt. Vor der Zeit der Oogonienentleerung sind die Paraphysen noch kurz und bestehen aus einer Reihe von 11-15 Zellen. Im Moment der Oogonienentleerung verlängern sie sich sehr rasch ohne weitere Vermehrung der Zellen, durchbrechen vermöge ihres gewaltigen Drucks die Aussenwand des Konzeptakels und wachsen dann geradlinig länger und länger. Wenn die Oogonien aus den eben gebildeten Öffnungen entleert werden, so verwirren sich die Paraphysen allmählich, indem sie sich um die Oogonien schlingen.

Bei der Keimentwicklung wird die Oosphäre zuerst durch eine Querwand in zwei beinahe gleiche Zellen geteilt, dann sondert sich eine linsenförmige, sogenannte Rhizoidzelle mit einer zweiten Wand, die mit der ersten parallel läuft, an einem Ende des Embryos ab.
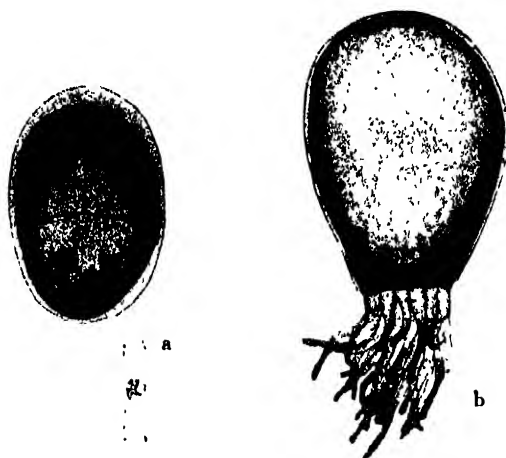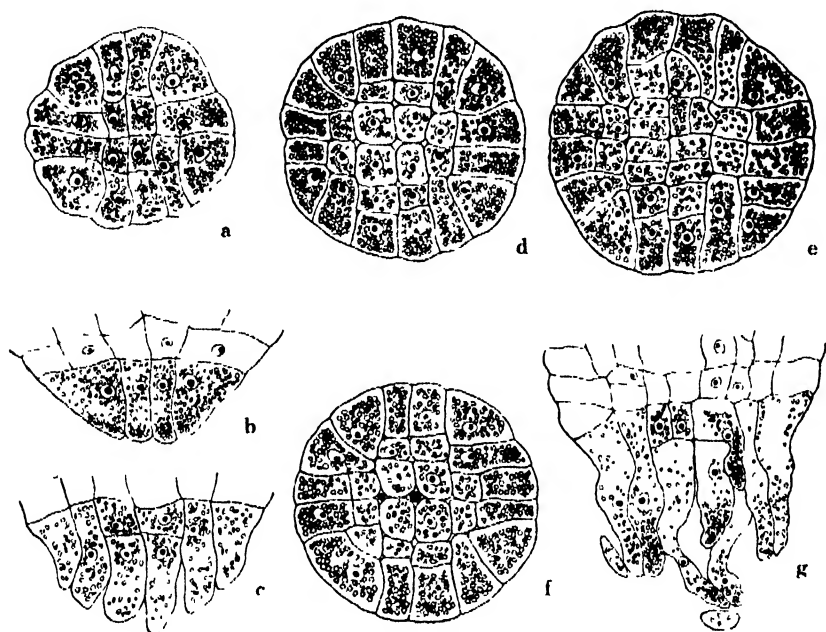

Text-fig. 1. *Cystophyllum sisymbrioides.*

a, Früheres Stadium der Keimentwicklung. Die Rhizoidzelle ist an dem untersten Ende differenziert und schon durch die erste Wand geteilt.

b, Ein späteres Stadium, etwa acht Tage nach Oogonienentleerung. Die Rhizoiden sind gut ausgebildet. ca. × 160.

Die Rhizoidzelle wird durch fünfmal aufeinander folgende Zellteilung in 32 Zellen geteilt. Aber es ist fast unmöglich, die Einzelheiten der Rhizoidenbildung am lebenden Material zu verfolgen, weil die Rhizoidzelle mit zahlreichen Körnern, die wahrscheinlich eine Art Ernährungsstoffe sind, erfüllt und sehr undurchsichtig ist. Deshalb habe ich gefärbte Mikrotomschnitte von ihr untersucht. Bis zur vierten Teilung der Rhizoidzelle stehen die Wände regelmässig immer auf einer der vorhergehenden Wände fast senkrecht. Text-fig. 2. a zeigt die Polansicht des 16zelligen Stadiums, wo die vierte Wandbildung eben vollendet und Text-fig. 2. b dasselbe Stadium in Längsschnitt. Die zentralen vier Zellen sind viel schmaler als die anderen. Die fünfte Teilung geht so vor sich, dass jede der zentralen vier Zellen merkwürdigerweise durch eine Querwand zweistöckig geteilt wird, während die anderen, an der Peripherie liegenden zwölf Zellen ihre neue Wand

in der gleichen Weise wie in den vorhergehenden Teilungen sich schaffen. Text-fig. 2. d bietet die Polansicht eines Querschnitts des die fünfte Teilung schon vollendet habenden Stadiums, wo 28 Zellen bemerkbar sind, aber im Längsschnitt kann man den zweistöckigen Zustand gut erkennen (Text-fig. 2. c).
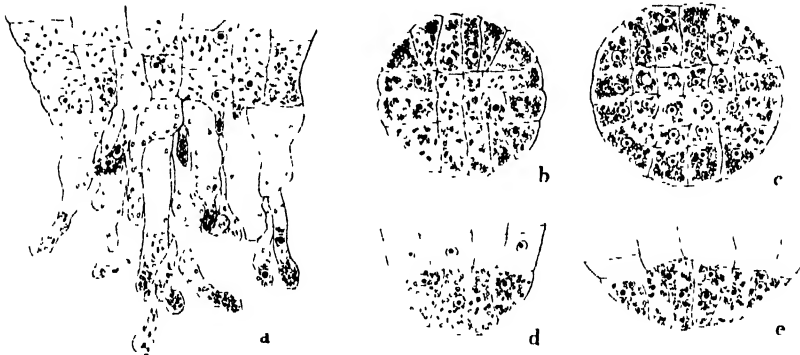

Text-fig. 2. *Cystophyllum sisymbrioides.*

a, Querschnitt des 16zelligen rhizoidalen Teils. b, Längsschnitt desselben Stadiums. c, Längsschnitt des 32zelligen Stadiums. Der zweistöckige Zustand ist zu sehen. d, Dasselbe Stadium im Querschnitt. e, Querschnitt des oberen Teils der Rhizoiden im noch fortgeschrittenen Stadium. f, Dasselbe Stadium im Querschnit des unteren Teils. g, Dasselbe Stadium im Längsschnitt. × 320.

Nach der fünften Teilung verlängern sich, mit Ausnahme der vier oberen Zellen im zweistöckigen Zustand, die 28 Zellen ohne weitere Zellteilung als Rhizoiden. Der Zustand des Embryos etwa acht Tage nach Oogonienentleerung ist in Text-fig. 1. b wiedergegeben. Die zentral liegenden Rhizoiden wachsen etwas schneller als die peripheren. In diesem Stadium wandert der Inhalt der Rhizoiden allmählich zu ihrer Spitze. Während die 28 Rhizoiden länger werden, vollzieht

sich in den vier oberen Zellen eine nochmalige Teilung, die mit der Längsachse des Embryos parallel verläuft, und jede Zelle von ihnen verlängert sich als ein Rhizoid, indem sie die Interzellularräume der benachbarten früheren Rhizoiden durchläuft (Text-fig. 2. e, f und g).

Etwa zwei Wochen nach der Oogonienentleerung veränderen sich die untersten, an die inneren Rhizoiden angrenzenden Leibeszellen; diese Zellen werden nämlich sehr plasmareich und verlängern sich allmählich zu Rhizoiden (Text-fig. 3. a).

In den oben beschriebenen Entwicklungsvorgängen der Rhizoiden kommen manchmal einige Unregelmässigkeiten vor, z. B. nach der fünften Teilung tritt zuweilen eine nochmalige Teilung in den an der Peripherie liegenden Zellen auf (Text-fig. 2. e, unten, rechts).

Text-fig. 3. a, *Cystophyllum sisymbrioides*. Langsschnitt des Embryos im weit spaterem Stadium, wo einige Rhizoiden von den Leibeszellen gebildet sind. b, c, d und e, *Cystophyllum Turneri*. Einige Stadien der Rhizoidenentwicklung. × 320.

Bei meinem Aufenthalt in Misaki konnte ich glücklicherweise auch einige Stadien der Rhizoidenentwicklung von *Cystophyllum Turneri* an auf die Küste getriebenem Material beobachten. Text-fig. 3. d, e und c stimmen je mit Text-fig. 2. b, c und d überein. Text-fig. 3. e vergegenwärtigt den zweistöckigen Zustand auch deutlich. Obwohl ich die weitere Entwicklung der Rhizoiden dieser Alge nicht verfolgen konnte, vermute ich, dass die Entwicklungsvorgänge wohl in der gleichen Weise wie bei *Cystophyllum sisymbrioides* vor sich gehen dürften. Die Oogonien und Keimlinge von *Cystophyllum Turneri* sind viel kleiner als die von *Cystophyllum sisymbrioides*.

An der Küste von Nordjapan wuchert eine andere Art von *Cystophyllum*, d. h. *C. hakodatense*. Ich werde bei nächster Gelegenheit diese Alge embryologisch studieren.

Nach TAHARAs Untersuchungen, die neuerdings durchgeführt wurden, werden die Rhizoidzellen von *Sargassum* und *Coccophora* durch drei aufeinander folgende Teilungen in acht Zellen geteilt, und in diesem Stadium beginnt die Rhizoidenbildung. Es ist eine interessante Tatsache, dass die dritten Wände bei *Sargassum Horneri* radial, dagegen bei *Coccophora Langsdorfii* auf den vorhergehenden ganz senkrecht gebildet werden. Also verlaufen bei der letzten Pflanze die Wandbildungen bis zur dritten Teilung ganz gleich mit *Cystophyllum*. Das Auftreten noch vielmaliger Teilungen der Rhizoidzelle bei *Cystophyllum* darf als Kennzeichen hoch differenzierter Organisation dieser Alge gelten.

Zum Schluss möchte ich Herrn Prof. Dr. NAOHIDE YATSU, Direktor der biologischen Station zu Misaki, und Herrn Prof. Dr. MASATO TAHARA meinen herzlichen Dank für ihre freundliche Unterstützung aussprechen.

den 25. September 1929.

LITERATURVERZEICHNIS

TAHARA, M. 1913. Oogonium liberation and the embryogeny of some Fucaceous algae. Journ. Coll. Sc. Imp. Univ. Tokyo, Vol. 32, Art. 8, pp. 1-13

TAHARA, M. 1928. Contributions to morphology of *Coccophora Langsdorfii* (TURN.) GREV. Sc. Rep., Ser 4, Biology. Tôhoku Imp Univ., Sendai, Japan. Vol. 3, No. 4, Fasc. 2, pp. 727 732.

TAHARA, M. 1929. Rhizoid formation in the embryo of *Turbinaria* (?) *fusiformis* YENDO and *Sargassum Thunbergii* O'KUNTZI Sc. Rep., Ser. 4, Biology, Tôhoku Imp. Univ., Sendai, Japan, Vol. 4, No. 1, Fasc. 1, pp. 1-6.

On the Number of Ganglion Cells in the Suprapharyngeal Ganglion and in the XXX. Ventral Ganglion of the Earthworm, *Pheretima megascolidioides* (GOTO and HATAI).

BY

HIDEOMI TUGE.

(Institute of Biology, Tôhoku Imperial University, Sendai).

I. INTRODUCTION.

In this investigation I tried to determine the number of ganglion cells in the suprapharyngeal ganglion and also in the XXX. ventral ganglion of the earthworm, *Pheretima megascolidioides* (GOTO and HATAI) (=Syn. *Perichaeta megascolidioides*).

OGAWA ('28) in this Institute already determined the number of ventral ganglion cells in the three other species (*P. communissima, P. hilgendorfi, P. vittata*) of *Pheretima*, but the suprapharyngeal ganglion has not yet been examined.

IMAI ('28) also published anatomical studies on distribution of the nerves arising from the cerebral ganglion and the ventral nerve cord, but no observations were yet made concerning the number of ganglion cells contained in these ganglia.

Since *Pheretima megascolidioides* is one of the largest earthworms measuring almost $1\frac{1}{2}$ feet when fully extended (HATAI, '24), and found commonly in Japan, and hence, owing to its larger body, this worm can be utilized for various physiological investigations, further data concerning the nervous organs seem highly desirable to possess.

II. MATERIAL AND METHODS.

The materials employed in the present investigation, were collected in the neighbourhood of the City of Imaharu in Shikoku. Of those specimens used for counting the ganglion cells the following body measurements were found:

No. of specimens.	Body length in cm. (under anaesthesia)	Body weight in g.
(A)	about 15	7.4
(B)	about 19.5	10.5
(C)	about 14.5	6.4
(D)	about 18	8.7

All the earthworms were adult, and were also in normal healthy condition.

The specimens were fully narcotized in keeping those in 20% alcohol for about 6 hours and the body was opened along the median dorsal line of the anterior part (specimen (A) and (B)). The pharynx was removed so as to expose clearly the circumpharyngeal connective ring. The circumpharyngeal connective ring was then cut on both sides and the suprapharyngeal ganglion was finally removed from the surrounding and supporting tissues.

The specimens, (C) and (D), were used for the purpose of enumeration of the number of ganglion cells in the XXX. segment. The reason for chosing the XXX. segment was firstly the relative simplicity of this segment with respect to the presence or absence of various organs and secondly to compare the present results with the number of ganglion cells counted by OGAWA ('28) on this same segment of three other species.

The ganglia were fixed in 80% alcohol and paraffine sections were made according to the usual procedure. The serial sections 10 micra thick were made and stained in 1% solution of toluidin blue in water, differentiated by anilin alcohol consisting of 1 vol. anilin and 9 vols absolute alcohol.

Instead of counting the cell bodies directly, the cell nucleolus was recorded as was practiced by HATAI ('02) and by OGAWA ('28). All necessary precautions were taken in avoiding possible mistakes of counting twice the same cell, owing to the frequent presence of the two nucleoli in one cell body, and so forth.

In fact I found 88 cases of presence of two nucleoli in the ganglion cells of specimen A and 68 cases in specimen B, while 41 cases in specimen C and 52 cases in specimen D, or on the average 0.8% of

the number of ganglion cells in the case of the suprapharyngeal ganglion and 3% in the case of the ventral ganglion. The latter ratio agrees entirely with that of OGAWA on the ventral ganglion cells of other species of *Pheretima.*

·The connective tissue cells or glia cells which are abundantly found both at the margin and among the ganglion cells, may be easily distinguished from the ganglion cells from the following chara·cteristics. In the connective cells, the cell membranes are indistinct and the nuclei alone are distinct. The nuclei are either ellipsoid or round in shape, and in the latter case they are usually deeply stained as the nucleoli, while in the ganglion cells the cell membranes are distinct and the nuclei appear clear containing less chromatic granules. The ganglion cells are usually larger than the connective tissue cells.

III. RESULTS OF THE ENUMERATION.

(1) *The number of ganglion cells in the XXX. segment.*

Specimen C

Number of the serial sections.	76.
Number of the ganglion cells.	1544.

Specimen D

Number of the serial sections.	57.
Number of the ganglion cells.	1588.

The number of ganglion cells in the XXX. segment is compared with that of the three other species of *Pheretima* (*P. communissima, P. hilgendorfi, P. vittata*) counted by OGAWA in the following table :

Author	TUGE	OGAWA ('28)		
Species	*P. megascolidioides*	*P. communissima*	*P. hilgendorfi*	*P. vittata*
No. of ganglion cells in the XXX. segment.	1566	1208	1171	1141

We clearly notice then that the number of ganglion cells in the XXX. segment of *Pheretima megascolidioides* is larger when compared

with the other *Pheretima*. An actual difference in the number of ganglion cells between the earthworm examined by me and the averages given by those species examined by OGAWA is 393 in favor of the former species. This difference of 393 ganglion cells is by no means large but since OGAWA's work shows astonishingly smaller individual variations in the number of ganglion cells, we may be justified to conclude that *Pheretima megascolidioides* possesses a larger number of ganglion cells in the XXX. segment when compared with the other three species under consideration. This difference appears to me due to the difference in the size of body of these worms.

As the present species is perhaps the largest *Pheretima* in Japan, naturally the surface of the segment should be much greater than in other smaller bodied worms and as the consequence the former species would require relatively larger number of the nervous supply which in turn demands correspondingly larger number of ganglion cells.

Findings of OGAWA that the presence of the prostate glands is associated with considerably larger number of ganglion cells in that segment when compared with the individual which belongs to the same species but happens to lack the prostate, seem to support the present view that normally the greater body surface would be associated with correspondingly greater number of ganglion cells and *vice versa*.

(2) *The number of ganglion cells in the suprapharyngeal ganglion.*

Specimen A

    Number of the serial sections. 80.
    Number of the ganglion cells. 9947.
Specimen B
    Number of the serial sections. 121.
    Number of the ganglion cells. 11640.

I have also counted in one case, specimen B, the number of ganglion cells in the ganglionic thickening corresponding to the enteric nerve (See, IMAI, Figs. 1, 3), and found 757 in it.

The number of ganglion cells in the suprapharyngeal ganglion was found to be 9949 and 11640 respectively or 10793 ganglion cells on the average.

From the work of IMAI, the number of nerves arising from the ventral ganglion is 4 pairs while the number of nerves arising from the suprapharyngeal ganglion is 10 pairs. The number of ganglion cells in the ventral ganglion is 1566 on the average while the number of ganglion cells in the suprapharyngeal ganglion is 10793 on the average. The ratio of the number of ganglion cells in the ventral ganglion with that in the suprapharyngeal ganglion is 1566 to 10793 or 1 : 6. On the other hand between the number of nerves arising from the ventral ganglion and those arising from the suprapharyngeal ganglion is 1 : 10 or 1 : 2,5.

From the above we note that while the number of nerves from the latter exceeds 2.5 times that from the former, the number of ganglion cells in the former exceeds 6 times that of the latter showing that the suprapharyngeal ganglion contains relatively a much greater number of ganglion cells in the suprapharyngeal ganglion than in the ventral ganglion when compared with the relative increase of the nerves.

Again we notice that in the ventral ganglion one pair of nerve is associated with 392 ganglion cells, while in the suprapharyngeal ganglion one pair of nerve is associated with 1079 ganglion cells, or in other words the pair of nerve in the suprapharyngeal ganglion is associated with nearly 2.5 times more cells than the pair of nerve in the ventral ganglion.

Whether or not this excess of ganglion cells is represented chiefly by the ganglion cells which give rise to the association fibers or this apparent excess merely indicates that each nerve involves more individual fibers in the suprapharyngeal ganglion than in the ventral ganglion, remains to be seen from the future investigation.

The mean value of the size of the ten largest ganglion cells found in four consecuting sections in the suprapharangeal ganglion, I found 27 micra in long axis, 18 micra in short axis while its nucleus gave 12 micra in long axis. The size of the smallest cell body was within 8 micra in long axis, its nucleus was about 5 micra respectively. Similar determination in the ventral ganglion showed that the largest cell body measures 30 micra in long axis and 25 micra in short axis having the nucleus of about 15 micra in long axis. The smallest gave

the size of 7 micra in long axis and its nucleus was about 5 micra in long axis.

According to OGAWA in *Pheretima vittata*, the larges ganglion cells have a dimeter of less than 18 micra and the nucleus of 12 micra, while the smallest have diameter more than 6 micra and the nucleus of 4 micra. These measurements of OGAWA thus differ considerably from these of mine. Whether this difference may be explained from also the difference of body size between the earthworms used, needs further investigation.

IV. SUMMARY.

(1) 10793 ganglion cells are found in the suprapharyngeal ganglion and 1566 ganglion cells in the XXX. ventral ganglion.

(2) Significance of these numbers when compared with the number of ganglion cells found in the three other species of *Pheretima* by OGAWA, and with the distribution of the nerves arising from the suprapharyngeal ganglion studied by IMAI, is discussed.

LITERATURE CITED.

1) HATAI, S. 1902. Number and size of the spinal ganglion cells and dorsal root fibers in the white rat at different ages. Jour. Comp. Neurol., Vol. 12, pp. 107-124.

2) HATAI, S. 1924. Reply to the remarks of Prof. WILHELM MICHAELSEN concerning the *Perichaeta megascolidioides*, GOTO and HATAI, and further observations made on this species on the relation of the body length to the number of segment and setae. Science Reports, Tôhoku Imp. Univ., Biology, Sendai, Japan. Vol. 1, pp. 23-44.

3) IMAI, T. 1928. Nervous system of the earthworm, *Perichaeta megascolidioides*, GOTO and HATAI. I. Gross anatomy of the nervous system. Science Reports, Tôhoku Imp. Univ., Biology, Sendai, Japan. Vol. 3, pp. 443-460.

4) OGAWA, F. 1928. On the number of ganglion cells and nerve fibers in some of the ventral nerve cord of the earthworm. I. The number of ganglion cells. Science Reports, Tôhoku Imp. Univ., Biology, Sendai, Japan. Vol. 3, pp. 745-756.

Effect of Anions in Chemical Stimulation in the Holothurian, *Caudina chilensis* (J. MÜLLER).[1]

By

Toshihiko Yamanouchi.

(Zoological Laboratory, College of Science, Kyoto Imperial University)

I. INTRODUCTORY NOTE.

It is well known that in the invertebrate animals cations are more effective than anions as chemical stimuli in neutral inorganic salts (Parker & Metcalf, 1906). In consequence of this, the proof of the differential stimulation of anions is generally more difficult than that of cations. In such case, the only method remaining for us is to increase the number of observations and to verify the experimental facts strictly, to determine whether they have a significance beyond the limit of errors.

The effects of anions of inorganic salts have been hitherto studied in various animals: in *Holothuria* (Crozier 1915), in *Ascidia* (Hecht 1918) in *Allolobophora* (Irwin 1918), in *Chiton* (Arey & Crozier 1919) and in *Chromodoris* (Crozier & Arey 1919). The salts used as stimulants by these authors are all potassium salts, which are the strong irritating reagents for invertebrate animals. Recently Loeb (1924 pp. 15, 129) proposed an opinion which denied the existence of the well known Hofmeister's lyotropic series, from his extensive works on colloid chemistry. But his conclusion was strongly opposed by some writers, for instance by Banctoff (1916, p. 340).

At present it is a problem of the first importance in the physiology of reception to ascertain whether such lyotropic effect exists or not in chemoreception for inorganic salts. In my previous paper, I reported on the experiments concerning the chemical stimuli in *Caudina chilensis* to some detail, and also touched briefly on the effect of anions (1929 pp. 94, 103). In this report the problem above mentioned is mainly discussed in the simplest case using the most common potassium salts.

The investigation was carried on in July and August, 1928, at the Asamushi Biological Station. I wish here to express my sincere thanks to Prof. HATAI for his kind direction and encouragement, and also to Assist. Prof. KOKUBO and Assist. TAMURA for their kind friendship throughout the course of the investigation.

II. METHOD.

Caudina is a Holothurian which dwells in the sand of the sea bottom near the station. The animals used in this experiment were all adult individuals of medium body size. The young or extremely old ones were avoided as material for experiment (compare YAMANOUCHI 1929, Table 21). The animal was collected just before the experiment, and was placed on a cloth spread over the edge of a glass jar of about 2 litre content. Only the portion to be stimulated was exposed to the air by the adjustment of the cloth, and after the stimulation it was immediately lowered into sea water.

Stimulation was effected by a *local* application of about half a c.cm. of the solution upon the middle region of the trunk (we distinguish the animal into two parts, the anterior bulky part which is called the *trunk* and the remainder posterior slender part which is called the *tail*). The animal responded to this local stimulus with the local stimulus with the local contraction of the muscles of the region stimulated (objectively the local depression of that part).

The interval between successive stimulations was about three minutes. The appearance of the body surface was always observed with care, and successive stimulation was continued only when the part previously

No. of specimen	KCl 0.2 M	K$_2$SO$_4$ 0.1 M	KNO$_3$ 0.2 M	KBr 0.2 M	KI 0.2 M
21	1	2	3	4	5
22	5	1	2	3	4
25	4	5	1	2	3
26	3	4	5	1	2
27	2	3	4	5	1
28	5	4	3	2	1
29	4	3	2	1	5
30	3	2	1	5	4
31	2	1	5	4	3
32	1	5	4	3	2

and so on.

stimlated regained its normal appearance after the stimulation. The part once stimulated was avoided in further stimulation. As a measure for the stimulating power of various reagents, the reaction time was measured with a stop watch. The order of reagent in stimulation was changed for each animal as in the above manner (the number is the order of stimulation).

Chemicals used were of following preparations;
hydrochloric acid (Merck's reagent), glycocoll (Kahlbaum, for enzymatic study), sodium chloride (Kahlbaum, for analysis), potassium chloride (Kahlbaum, for analysis with garant table), sulphate, nitrate, bromide and iodide of potassium (Merk's extra pure crystal).

Caudina is reactive to more highly hypertonic solutions than its environment, but not reactive to hypotonic solutions, including distilled water (YAMANOUCHI 1929 p. 91). So it goes without saying that the solution used in these experiments gave no osmotic stimuli to the animal.

III. EFFECT OF NON-IONIZED MOLECULES

In comparing the stimulating effect of electrolytes, the most important factor is the concentration of ions. The non-ionized part, however, must not be omitted from consideration. CROZIER (1916) pointed out the presence of the potentially ionizable hydrogen within undissociated acid molecules. For the purpose of comparing the effect of the non-ionized part, the same kind of buffer solution is to be compared as adopted by HARVEY (1920).

In *Caudina* it was shown that the weaker acid or alkali is more effective as a stimulant than the stronger one when they contain approximately equal hydrogen ions (1929, pp. 96-97). In the summer of 1928, acid stimulation was reexamined, using SÖRENSEN's hydrochloric acid and glycocoll mixture. The mixture was prepared by mixing 97.4 c.cm. of 0.1 normal HCl solution with 102.6 c.cm. of 0.1 molar glycocoll and NaCl solution. This mixture had pH value of 2.1 at 25°C. (I thank Mr. KOBAYASHI for his kindness in measuring the pH value electrometrically by chinhydron-electrode). This was diluted with distilled water and the ranges having equal hydrogen ion concentration were sought by a colorimetric method (as an indicator thymol blue after CLARK was used), for glycocoll mixture

pH		dilution		pH
2.1	=	$\frac{10}{10}$		
2.1	$\leqq$	$\frac{8}{10}$	<	2.2
2.1	<	$\frac{7}{10}\cdots\frac{4}{10}$	<	2.2
2.1	<	$\frac{3}{10}$	$\leqq$	2.2

and for hydrochloric acid

2.1	=	0.01 N		2.2
2.1	$\leqq$	0.009 N	<	2.2
2.1	<	0.008 N	<	2.2
2.1	<	0.007 N	$\leqq$	2.2

Thus glycocoll mixture diluted to $\frac{4}{10} \frac{7}{10}$ and 0.008 N HCl solution were prepared to have pH value, 2.15±0.05 at 25°C.

TABLE 1.

Effect of Hydrogen ions.
Five solutions are of equal pH value, 2.15±0.05 at 25°C.

		Reaction time in second			
	HCl 0.008 N[1]	HCl-glycocoll mixture, diluted to
		$\frac{4}{10}$	$\frac{5}{10}$	$\frac{6}{10}$	$\frac{7}{10}$
Total acid	0.008 N	0.0195 N	0.0244 N	0.0292 N	0.0341 N
Ranges	1.9–3.8	2.0–3.5	1.8–2 9	1.7–2 9	1.4–2.9
Means[2]	2.68 ±0.069	2.60 ±0.060	2.37 ±0.042	2.26 ±0.061	2.18 ±0.070
Standard deviation[3] (S. D.)	0.445 ±0.049	0.400 ±0.043	0.282 ±0.030	0.402 ±0.043	0.466 ±0.050
Temperature (t) in °C	23.5—25.5
Number of animal	20

1) N denotes a normal solution.

2) The probable error of the mean is calculated from $0.6745 \cdot \frac{S.D.}{\sqrt{n}}$

3) The standard deviation and its probable error are calculated from $\sqrt{\frac{S(v^2)}{n-1}} \pm 0.6745 \cdot \frac{S.D.}{\sqrt{2n}}$, where n is the number of observation and $S(v^2)$ is the sum of the square of the residual.

For each animal five successive stimulations were accomplished, and the results are summerised in Table 1. It is clear that the non-ionized part of the acid molecules exerts an additional effect in stimulation. In HARVEY's study (1920) the intensity of taste was emploeyd as a criterion for comparison. The reaction times and the magnitude of reaction are intimately related as indicated by the effect of dilution in chemical stimulation (YAMANOUCHI 1929, p. 99). The conclusion of HARVEY is correct when the reaction times are employed as a criterion for stimulating effect.

III. STIMULATION BY KCL SOLUTION

The arithmetic means of reaction time are usually employed as a criterion for the differential sensitivity of animal to various stimulants. The determination of the limit of difference from which different stimulants are put into the order, is the most important thing, but it is at the same time a matter in which the author may be utterly arbitrary, *if* he has no grounds for determining the limit.

In Table 2 reaction times obtained from 26 animals are listed. The mean values show apparently as a whole a gradual increase in successive stimulation.

TABLE 2.

Successive stimulation by 0.2 M[1] KCl solution. For each animal five successive stimulations (I– V) are made at 3 minute intervals. Number of animal is 26.

Date Aug.	t	No.[2]	Reaction time in second				
			I	II	III	IV	V
16	27	41	2.2	2.1	2.1	2.2	2.3
		42	1.8	2.2	2.2	2.0	1.8
		43	2.0	2.2	2.2	1.8	1.8
		44	1.6	1.8	1.8	1.9	2.0
		45	2.0	2.0	1.8	2.0	1.9
		46	2.3	2.6	2.0	2.2	2.2
		47	2.6	2.4	2.4	2.4	2.4
		48	2.5	2.4	2.2	2.8	2.2
		49	2.6	2.7	2.5	2.4	2.5
		50	2.6	2.8	2.6	2.6	3.0

1) M is a molar solution.
2) No. is the number of specimen.

Date Aug.	t	No.	Reaction time in second				
			I	II	III	IV	V
18	25	51	2.4	2.0	2.2	2.0	2.1
		52	2.0	2.2	2.1	2.0	1.9
		53	1.7	2.0	1.8	2.2	2.0
		54	1.6	1.6	1.7	1.6	1.8
		55	1.8	1.8	2.0	2.0	1.8
		56	1.8	1.9	1.9	1.7	1.8
		57	1.8	1.9	2.1	2.0	1.7
		58	1.9	1.8	2.0	2.1	1.8
19	23.5	59	2.2	2.4	2.3	2.3	2.2
		60	2.1	1.9	2.0	1.8	1.8
		61	2.6	2.6	2.3	2.3	2.4
		62	2.8	2.8	2.4	2.6	2.5
30	26.5	112	2.2	1.9	2.1	2.0	1.9
		113	1.8	2.0	2.0	1.8	2.0
		114	2.2	2.0	2.0	1.8	2.0
		115	1.8	2.0	2.0	2.0	2.0
Ranges			1.6–2.8	1.6–2.8	1.8–2.6	1.6–2.6	1.7–3.0
Means			2.11 ±0.047	2.15 ±0.045	2.10 ±0.030	2.10 ±0.039	2.07 ±0.040
S. D			0.352 ±0.033	0.342 ±0.033	0.224 ±0.021	0.295 ±0.028	0.304 ±0.029

So it becomes necessary to rearrange the reaction times and then to calculate the mean values from the new table for the purpose of eliminating the after effects due to the former stimulus. Table 3, rearranged in the same manner as described in Method II, shows no such effect among the means.

TABLE 3.

Successive stimulation by 0.2 M KCl solution (continued). Rearranged from the data given in the Table 2. The method of rearrangement is explained in the text. The letters a – e merely designate the columns

	Reaction times in seconds				
	a	b	c	d	e
Ranges	1.7–2.7	1.6–3.0	1.7–2.8	1.6–2.6	1.6–2.8
Means	2.12 0.038	2.09 0.046	2.12 0.035	2.08 0.038	2.12 0.043
S. D.	0.285 0.027	0.351 0.033	0.272 0.025	0.288 0.027	0.324 0.030

The difference between the largest and the smallest means are (taking the case of the maximal probable error)

$(2.12 + 0.046) - (2.08 - 0.046) = 2.166 - 2.034 = 0.132,$

or there is a difference of ±0.066 second on both sides of the mean. From this, the apparent activation by successive stimulation shown in Table 2 seems to be not a decisive one.

IRWIN (1918) reported the initial acceleration on successive stimulation of the earthworm by 0.1 M KCl solution. In IRWIN's experiment, the same portion was successively stimulated. In *Caudina* the portion stimulated was renewed in each stimulation. Therefore the absence of the initial acceleration is rather natural.

TABLE 4.

Successive stimulation by 0.2 M KCl solution (continued). Means and standard deviations are calculated for each animal from the reaction time given in the Table 2.

No.	Ranges	Means	S. D
41	2.1-2.3	2.18±0.025	0.084±0.018
42	1.8-2.2	2.00±0.060	0.100±0.043
43	1.8-2.2	2.00±0.060	0.200±0.043
44	1.6-2.0	1.82±0.045	0.148±0.032
45	1.8-2.0	1.94±0.027	0.089±0.019
46	2.0-2.6	2.26±0.066	0.219±0.047
47	2.4 2.6	2.44±0.027	0.089±0.019
48	2.2-2.8	2.42±0.074	0.249±0.053
49	2.4-2.7	2.54±0.032	0.114±0.024
50	2.6-3.0	2.72±0.054	0.179±0.038
51	2.0-2.4	2.14±0.051	0.167±0.036
52	1.9 2.2	2.04±0.034	0.114±0.024
53	1.7-2.2	1.94±0.063	0.207±0.044
54	1.6-1.8	1.66±0.027	0.089±0.019
55	1.8-2.0	1.88±0.033	0.110±0.023
56	1.7-1.9	1.82±0.025	0.084±0.018
57	1.7-2.1	1.90±0.048	0.158±0.034
58	1.8-2.1	1.92±0.039	0.130±0.028
59	2.2-2.4	2.28±0.025	0.084±0.018
60	1.8-2.1	1.92±0.039	0.130±0.028
61	2.3-2.6	2.42±0.046	0.152±0.032
62	2.4-2.8	2.62±0.054	0.179±0.038
112	1.9-2.2	2.02±0.039	0.130±0.028
113	1.8-2.0	1.92±0.033	0.110±0.023
114	1.8-2.0	2.00±0.043	0.141±0.030
115	1.8-2.0	1.96±0.007	0.089±0.019
Total*	1.6-3.0	2.11±0.017	0.290±0.012

* Means and S. D. for the total are calculated from 130 total measurements.

The standard deviation (S. D.) in Table 4 is calculated for each animal from five reaction times. This is a measure for the error of single observation for each animal. No. 46 and 48 give extraordinarily large S. D., which seems to be due to a mistake of the observer. The S. D. given in each column in Tables 1–7 (excepting 4) includes two factors, that is, the error for each measurement as indicated in S. D. of the Table 2 and the degree of individual difference.

From the Tables 2 and 3, it is clear that by the same stimulant approximately equal reaction times are obtained. In other words, by the same concentration of ions, the same effect is expected. Increasing the concentration of the solution, the concentration of ions are increased, and the reaction times decreased thereby (Table 5).

TABLE 5.

Effect of concentration of KCl solution.

| | Reaction time in second | | | | |
	0.18 M	0.19 M	0.20 M	0.21 M	0.22 M
Ranges	1.9–4.4	1.6–3.9	1.8–3.2	1.6–3.0	1.4–3.0
Means	2.77 ±0.095	2.50 ±0.085	2.35 ±0.064	2.24 ±0.058	2.17 ±0.058
S. D.	0.633 ±0.068	0.563 ±0.060	0.422 ±0.045	0.387 ±0.041	0.387 ±0.041
t	24.5 –27.5				
Number of animal	20				

IV. EFFECT OF ANIONS.

The results obtained by five potassium salts in equivalent normal solution is given in the Table 6. In which, nitrate and sulphate, especially sulphate, are inferiorly effective compared with the other three halogen compounds. Iodide is the most effective. The threshold concentrations of these salts for chemical stimulation showed the same tendency (YAMOUCHI 1929, p. 93). However, sulphate and nitrate are known as less ionizable than the other halides. The true effect should be compared with the solutions containing equal concentration

of ions.   According to the mordern theory concerning the dissociation of electrolytes, total dissociation occurs in aqueous solution of strong electrolytes.   Even so, the most important point is the activity of ions.

TABLE   6.

Effect of anions of five potassium salts.

	Reaction time in second				
	KCl 0.2 M	K_2SO_4 0.1 M	KNO_3 0.2 M	KBr 0.2 M	KI 0.2 M
Ranges	1.8–2.9	2.4–3.4	2.0–2.8	1.8–2.9	1.8–2.6
Means	2.30 ±0.038	2.95 ±0.046	2.41 ±0.041	2.28 ±0.037	2.15 ±0.034
S. D	0.261 ±0.027	0.318 ±0.032	0.283 ±0.029	0.258 ±0.026	0.233 ±0.024
t			25—27		
Number of animal			22		

From the data of stoichiometry and the electrolytic conductivity we know that in sulphate and nitrate the activity of ions is inferior to the other halides.   To check this point, the concentration of these two salts should be raised to some extent.

TABLE   7.

Effect of anions of five potassium salts

	Reaction time in second				
	KCl 0.2 M	K_2SO_4 0.126 M	KNO_3 0.213 M	KBr 0.199 M	KI 0.197 M
Ranges	1.8–3.2	1.9–3.2	1.9–3.0	2.0–3.2	1.9–2.8
Means	2.45 ±0.059	2.51 ±0.049	2.37 ±0.043	2.46 ±0.049	2.32 ±0.041
S. D.	0.408 ±0.041	0.337 ±0.034	0.299 ±0.030	0.340 ±0.035	0.285 ±0.029
t			25—27		
Number of animal			22		

At present we do not practically know an exact method of preparing the solutions in which the number of the activated ions can be kept constant in these potassium salts. The lack of an appropriate method forced me to adopt the following procedure : -- from the data on the equivalent conductivity at 18°C (given in LANDOLT-BERNSTEIN's Tabellen, excepting the column $\frac{1}{2}K_2SO_4$) the dissociation degrees are calculated, and inverse-proportionally to this, the concentrations of the five potassium compounds in the Table 7 were calculated, such that

	KCl	KBr	KI	KNO$_1$	$\frac{1}{2}K_2SO_4$[1]
equivalent $\quad\int 0$	130.1	132.3	131.1	126.5	133.0
conductivity $\{0.2$ M	107.96	110.4	110.5[2]	98.74	87.7
degree of dissociation	0.830	0.834	0.844	0.781	0.659
reciprocal of the above	1.205	1.199	1.185	1.280	1.517
,, $\times \dfrac{0.2}{1.205}$	0.2	0.199	0.197	0.213	0.252

(1) Cited from LEWIS' System of Physical Chemistry Vol. I. 1921.

2) Calculated by interpolation.

From the Table 3, there is a probability that the difference in the mean reaction time reaches ±0.066 second when the number of observation is 26. This probable error must be increased when the number of observation is 22 as in the Tables 6 and 7. From the table given by PEARSON (1914, p. 529) the ratio of the standard deviation in both cases becomes (S. D.) n=22/(S. D.) n=26 = 1.0056. The probable error required is $1.0056 \times \dfrac{\sqrt{26}}{\sqrt{22}} \times 0.066 = 0.072$. Therefore we should apply ±0.072 second in the case when the number is 22.

Now we must adopt this new probable error, ±0.072 second, instead of that given in the Tables 6 and 7, as the former is more natural and also includes all the cases of the probable errors within its limit. If this assumption is correct, the stimulating effect among the five potassium salts in an equivalent molar solution (0.2 N) is in the following decreasing series,

KI $\geqq$ KBr, KCl, KNO$_3 > \frac{1}{2}K_2SO_4$
KI > KCl   . . . . (a)
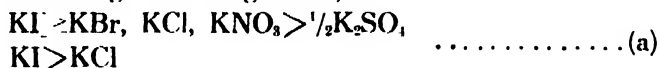
Now the question arises as to the rôle of the non-ionized part of the

molecules (compare the Table 1). In the Table 7, the molar concentration of KCl, KBr, KI are so close together that the effect due to the non-ionized molecules might be of the same order. On the contrary, the stimulating effect of KNO_3 and K_2SO_4 should be considered somewhat postponed. From such consideration we have the following order among the solutions with approximately equal ionic concentration (Table 7),

KI≶KNO₃, KCl, KBr ≷K₂SO₄
............(b)
KI>K₂SO₄
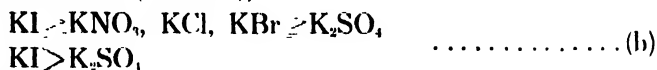
These series are analogous to the well known HOFMEISTER's lyotropic series (HÖBER 1926 p. 224).

Although these five potassium salts stand side by side in their stimulating effect, it is sure that among them some differences in the effect of stimulation exist beyond any probable errors of the experiment.

V. DISCUSSION.

The stimulating effect of potassium salts, when they are applied locally to certain invertebrate animals is summarised in the following table,

Animal	Method	Concentration	Order	Author
Holothuria	reaction time	0.1 N	Cl⁻>SO₄	CROZIER, 1915
Ascidia	limiting concentration		I>Br⁻ Cl⁻·NO₃	HECHT, 1918
Allolobophora	reflex time	0.1—0.3 N	Cl⁻·NO₃	IRWIN, 1918
Chiton	time occupied by gill reaction	$\frac{5}{8}$ N	Cl⁻ NO₃>Br⁻ I	ARRY & CROZIER, 1919
Chromodoris	amplitude of reaction	$\frac{5}{8}$ N	Cl=Br=I—NO₃	CROZIER & ARRY, 1919
Caudina	reaction time	0.2 N	I≷Br, Cl, NO₃>SO₄	

In these cases, the reaction given by *Chiton* is much different from the other animals. In *Chromodoris,* four salts give an equal effect. But such a simple method as to observe directly the amplitude of reaction might not give a decisive result In *Holothuria, Ascidia, Allolobophora,* and *Caudina,* the orders are proportional to the degree of ionization of these salts. Then it becomes an urgently necessary

problem to accomplish the experiments with solution having equal concentration of ions. It is possible to presume the effect of anions from the data obtained from the solutions with equivalent molar concentration. Still in such a case it is a questionable problem how we can estimate the activity of ions of these salts. As we have at present no precise method for preparing such solutions which have equivalent ionic concentrations in these salts, I can not pretend that the method of preparing the solution given in Table 7 is an exactly appropriate one. However, the result given in the Table 7 seems to me to tell the actual rôle of the anions, more than that given in the Table 6.

There shall be no question as to the fact that the chemical stimulation by inorganic salt is accomplished by the ions surrounding just the surface of receptor cells. In this case the first important factor for stimulation is the activity of ions. The other part of the ions which are not activated exert an additional effect in stimulation. The existence of the lyotropic effect concerning the stimulating effectiveness of potassium salts throws some light on the solution of the mechanism of chemical stimulation. But how the effect of anions is related is a problem which requires further study.

VI. SUMMARY.

1. The conclusion of HARVEY as to the effect of the non-ionized part of the molecules concerning the human chemical sensation has been proved to be right in chemical stimulation of *Caudina chilensis*.

2. Five salts of potassium are not equal in their effectiveness in chemical stimulation. They are arranged in the decreasing series in their strength in stimulation as follow

in 0.2 N solution

$KI \geqq KBr, KCl, KNO_3 > \frac{1}{2}K_2SO_4$

$KI \smile KCl$

in the solution approximately with equal ion concentration,

$KI \geqq KNO_3. KCl, KBr \geqq K_2SO_4$

$KI > K_2SO_4$
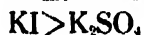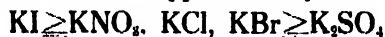
VII. LITERATURE CITED.

1) AREY, L. B. & CROZIER, W. J. 1919. The sensory responses of *Chiton*. J. Exp. Zoöl., Vol. 29, p. 242.

2) BANCROFT, W. D. 1926. Applied Colloid Chemistry. New York. p. 340.

3) CROZIER, W. J. 1915. The sensory reactions of *Holothuria surinamensis* LUDWIG. Zool. Jahrb., Abt. allg. Zoöl. u. Physiol., Bd. 35, p. 233.

4) CROZIER, W. J. 1916. The Taste of Acid. J. Comp. Neurol., Vol. 26, pp. 453–462.

5) CROZIER, W. J. & AREY, L. B. 1919. Sensory reactions of *Chromodoris zebra*. J. Exp. Zoöl., Vol. 29, p. 302.

6) HARVEY, R. B. 1920. The relation between the Total Acidity, the Concentration of the Hydrogen Ion, and the Taste of Acid Solutions. J. Amer. Chem. Soc., Vol. 42, pp. 712–714.

7) HECHT, S. 1918. The Physiology of *Ascidia atra* LESUEUR. II. Sensory physiology. J. Exp. Zoöl., Vol. 25, p. 261.

8) HÖBER, R. 1926. Physikalische Chemie der Zelle und der Gewebe. Leipzig. p. 224.

9) IRWIN, M. 1918. The nature of sensory stimulation by Salts. Amer. J. Physiol., Vol. 47, p. 276.

10) LOEB, J. 1924. Die Eiweisskörper. Berlin. pp. 15, 129.

11) PEARSON, K. 1914. On the distribution of the standard deviations of small samples. Biometrika, Vol. 10, p. 529.

12) YAMANOUCHI, T. 1929. Notes on the Behavior of the Holothurian, *Caudina chilensis* (J. MÜLLER). Science Reports, Tôhoku Imp. Univ., IV. Series (Biology), Vol. IV. pp. 89–106.

Carbon Dioxide Production in Relation to the Growth of Body of Earthworm, *Pheretima communissima* GOTO et HATAI.

BY

TERUHEI HINO.

(Biological Institute, Tôhoku Imperial University, Sendai).

INTRODUCTION.

There is a great amount of literature concerning the growth of vertebrates, especially that on the mammals; but very little has been done on the growth of invertebrates, of which I may enumerate the following few representative researches:— LUCIANI and LO MONACO ('97), OSTWALD ('08), and YAGI ('26) on the growth of silk-worms, STRAUS ('11) on honey-bee, and EIDMAN ('24) on *Dixippus*.

So far as I am aware, no studies have been made on the growth of any annelids, and therefore, as the first step in furnishing much needed data on the growth of the body of this interesting group of invertebrates, the present research on the growth of the Japanese earthworm was undertaken.

I wish to express my hearty thanks to Prof. S. HATAI for his valuable suggestions and criticism throughout this work, and to gratefully acknowledge the aid given me by the late Assist. Prof. K. OKAZAKI.

MATERIAL AND METHOD.

Pheretima communissima, GOTO et HATAI, which is very commonly found in Northern Japan, was used for the present investigation. As the first step the cocoons were collected during early spring and reared in the laboratory. Each cocoon was kept separately in a small glass tube which was filled with the soil where the cocoons were found. Out of the fifty cocoons seventeen were hatched out on the third of April, but only 12 young worms just hatched were kept for studying the growth phenomena. Newly hatched worms were transferred separately into a small glass dish (60 mm. in diameter

75 mm. in depth), which was filled to one-third of its entire depth with the soil from the glass tube. In order to prevent the escape of the worms from the dish and also to preserve the proper moisture, the soil was covered with water-plants or with clover. When the earthworms had grown sufficiently large, about 60 mm. in length, they were transferred to a large dish (200 mm. in diameter 75 mm. in depth).

The normal growth of the earthworms in the laboratory necessitates frequent change of soil in the dish with fresh soil from the field where the earthworms are living; and it is also necessary to keep the soil properly moistened. It was found that when the fallen clover leaves in the dish were half decomposed they were eaten by the earthworms. Immediately after hatching, the worms were weighed and the length measured, and this procedure was continued once every week throughout the entire course of the experiment. Before taking the measurements the worms were washed and the excess of water on the body surface was removed gently with filter paper. In the body weight is included the content of the alimentary tract.

GROWTH OF EARTHWORM.

In Table I are given the data on the growth of the *Pheretima communissima* which were raised in the laboratory.

TABLE I.

Weeks	No. of specimens	Average weight in grams.	Average length in mm.	Diameter, computed from $(r=\sqrt{\frac{W}{\pi l}})$ in mm.	Body surface (computed) $(S=2\pi rl)$ in sq. cm.	Body surface computed $(S=10\sqrt[3]{W^2})$
(III. 10. cocoon)		0.036				
(IV. 3 hatch out)	6	0.031	29	1.16	1.04	0.98
1		0.049	41	1.23	1.58	1.34
2		0.082	45	1.52	2.15	1.89
3		0.160	54	1.92	3.39	2.95
4		0.214	61	2.12	4.05	3.58
5		0.322	72	2.34	5.28	4.70
6		0.492	84	2.72	7.17	6.23
7		0.759	97	3.16	9.64	8.32
8		0.876	106	3.24	10.80	9.15
9		0.910	108	3.28	11.10	9.39

Weeks	No. of speci-mens	Average weight in grams.	Average length in mm.	Diameter, computed from $\left(r=\sqrt{\dfrac{W}{\pi l}}\right)$ in mm.	Body surface (computed) $(S=2\pi rl)$ in sq. cm.	Body surface computed $\left(S=10\sqrt[3]{W^2}\right)$
10		0.952	109	3.34	11.43	9.68
11		1.035	111	3.46	12.06	10.4
12		1.635	127	4.06	16.17	13.9
13		1.858	132	4.24	17.57	15.1
14		2.021	138	4.32	18.61	16.0
15		2.759	152	4.83	22.78	19.7
16		3.506	167	5.18	27.08	23.0
17		3.932	167	5.48	28.73	24.9
18		4.004	167	5.52	28.94	25.2

As is shown in Table I, the earthworm immediately after hatching shows on the average 29 mm. in length and 0.031 grams in body weight.

Eighteen weeks after hatching the worm measures 167 mm. in length and 4.004 grams in body weight and is considered fully matured. During these 18 weeks the earthworms then showed an increase of (167–29)/18 mm. or 7.7 mm. in body length and (4.004-0.031)/18 grams or 0.022 grams in body weight per week.

In Fig. 1 the curves showing the growth of body in weight and in length with respect to their corresponding ages in weeks are given.

The general features of these two curves resemble each other, indicating that a similar growth rate between these two characters is maintained up to 16 weeks. After 16 weeks no further noticeable increase is shown in length, though a slight continuation of increase is shown in weight.

In growing animals it is a general rule that the rate of growth decreases with increasing age. The same tendency is also shown in the growth of the earthworms, though a considerable irregularity is noted in the later stage, as will be seen in Table II, which shows the values calculated from the data given in Table I, as well as in Fig. 3. The absolute amount of increment is also shown in Fig. 2 for further reference.

Considerable fluctuations which occur at a later period, or at about eleven weeks of age, are due to the sudden development of the clitellum and of the generative organs. However this sudden increment due to greater development of the reproductive organs is a temporary phenomenon of periodical nature, and thus cannot be considered in a
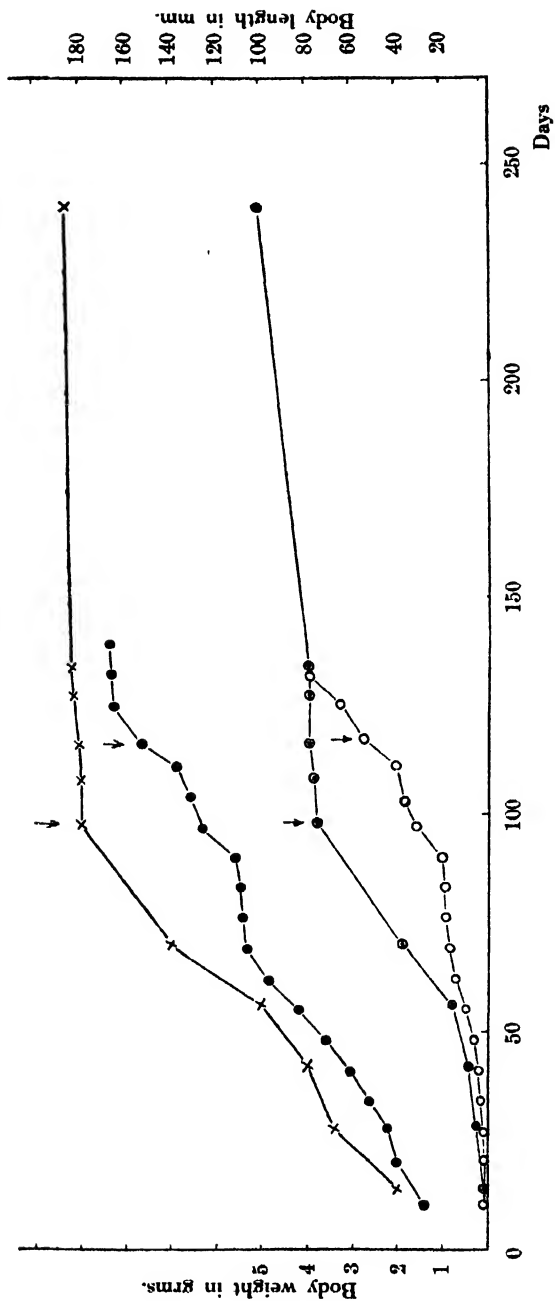
Fig. 1. Showing the growth of earthworms.

$-\times-\times-$ Body length $\Big\}$ grown in field.
$-\bullet-\bullet-$ Body weight $\Big\}$

$-\bullet-$ Body length $\Big\}$ grown in laboratory.
$-\circ-\circ-$ Body weight $\Big\}$

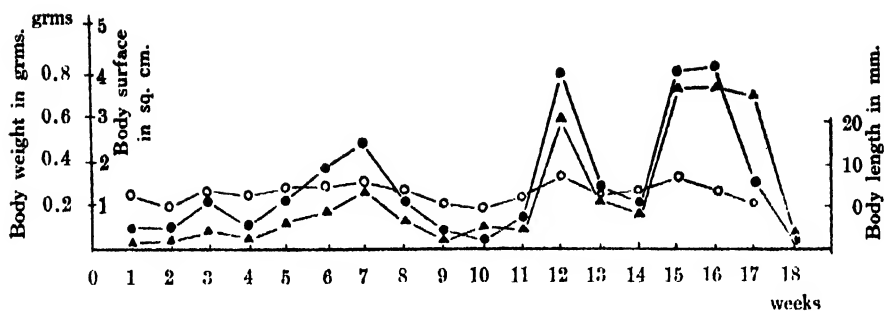Arrows showing the period clitellum was formed.

Fig. 2. Showing the absolute increment.

—·▲·· ▲—   Body weight.   —●——●·· ·   Body surface.
— ○——○——   Body length.
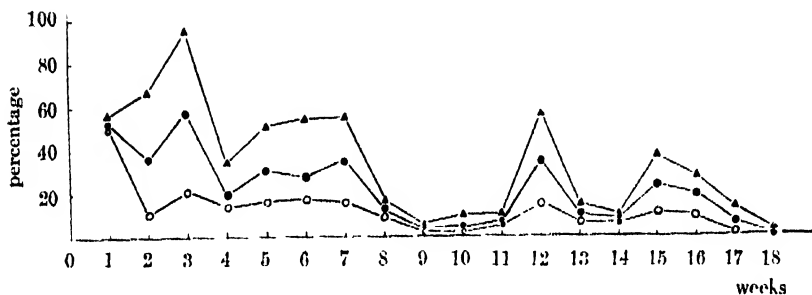
Fig. 3. Showing the percentage of increment.

·—▲   ▲ —   Body weight.   —●——●——   Body surface.
—·○   ○——   Body length.

strict sense as the growth of the body. If we therefore disregard
this temporary increment due to the sexual cycle, the growth rate
decreases regularly with the age of the earthworms as in other animals
of which the growth was followed.   Since the sudden development of
the sex organ manifests itself chiefly by the increased weight· owing
to sudden accumulation of the formed gonads, and since the body
length itself may not participate in such change, we should anticipate
a less degree of fluctuation in the relative daily increment in the
latter character.   As is shown in Table II as well as in Fig. 3, we
actually notice that our anticipation just stated in this regard is well
realized, indicating that the greater variability at the later period was
due to the accumulation of the sexual products.

TABLE II.

Weeks	Increments of weight.		Increments of length.		Increments of surface.	
	absolute in grams	%	absolute in mm.	%	absolute in sq. cm.	%
0	0.018	58.2	12	50.6	0.54	51.9
1	0.033	67.3	4	9.7	0.57	36.0
2	0.078	95.1	9	20.0	1.24	57.6
3	0.054	33.7	7	12.9	0.66	18.9
4	0.108	50.4	11	16.3	1.23	30.4
5	0.170	52.9	12	16.6	1.89	28.2
6	0.267	54.2	13	15.5	2.47	34.2
7	0.117	15.4	9	9.3	1.16	12.0
8	0.042	4.5	2	1.9	0.30	2.8
9	0.076	8.6	1	0.9	0.33	3.0
10	0.083	8.7	4	3.7	0.63	5.5
11	0.600	58.0	16	14.4	4.11	33.9
12	0.223	13.0	5	3.9	1.40	8.7
13	0.163	8.6	6	4.5	1.04	5.9
14	0.738	36.5	14	10.0	4.17	24.4
15	0.747	27.0	15	9.8	4.30	18.8
16	0.426	12.1	0	0.0	1.65	6.0
17	0.072	1.8	0	0.0	0.21	0.7

Thus far I have described the growth of body of the earthworms which were artificially raised in the laboratory under laboratory conditions, but it is a well known fact that the growth of the body is largely influenced by various external factors, such as an abundance of food, proper moisture, temperature, etc., and thus the growth rate as well as the general form of the growth curves obtained from the artificially raised worms may not be the same with worms grown naturally. In order to get some information regarding the growth of the earthworms which were grown under natural conditions, I have carried out the following test. From the chosen ground in the field where the cocoons were collected newly hatched worms were collected, and then twice every week worms were collected from the same ground, and the successive growth of the newly collected specimens was studied.

The results of the measurements made on usually more than fifteen worms in each test are shown in Table V and in Fig. 1. As is clear from the curves, the growth of the earthworms collected from the field is much faster than of those reared in the laboratory. Indeed the former reach the full-grown stage at about 14 weeks instead of 18 weeks, as in the latter specimens. The clitellum is also formed at an earlier period in the field grown earthworms than in the labora-
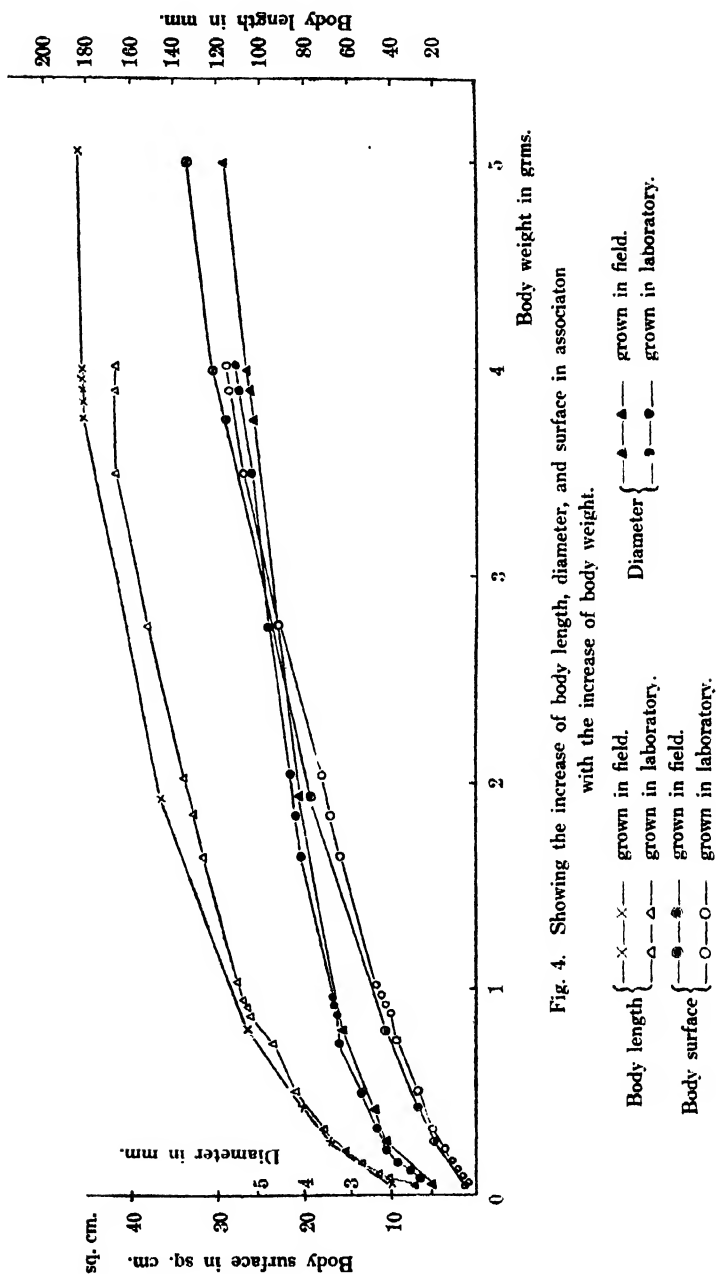
Fig. 4. Showing the increase of body length, diameter, and surface in associaton with the increase of body weight.

Body length $\left\{\begin{array}{l} \text{—×—×—} \\ \text{—△—△—} \end{array}\right.$ grown in field.
grown in laboratory.

Body surface $\left\{\begin{array}{l} \text{—●—●—} \\ \text{—○—○—} \end{array}\right.$ grown in field.
grown in laboratory.

Diameter $\left\{\begin{array}{l} \text{—▲—▲—} \\ \text{—,—●—} \end{array}\right.$ grown in field.
grown in laboratory.

tory raised ones, though the final body weight at 14 weeks of the former is nearly the same as that at 18 weeks of the latter. The body lengths at these two respective ages are a little greater in the field grown than in the laboratory grown worms.

We therefore conclude that the velocity of growth rate was more rapid in the earthworms grown naturally than those raised in the laboratory.

This difference in the growth rate between the field and laboratory grown earthworms may depend upon the relative richness of nourishment as well as upon a more proper degree of moisture in the former than in the latter.

We may say also that in earthworms the adequacy of nourishment not only accelerates the growth rate but also the period for attainment of sexual maturity. In other words, the better the nourishment the earlier is the period of maturity. The truth of this statement is well shown in Fig. 1 where the two growth curves are compared with each other.

As was stated already, the growth of body in weight and in length differs slightly between the field and laboratory grown worms for a given same age, and the former reaches maturity in a shorter period than the latter, though the final maximal weights, as well as lengths, are nearly identical.

The growth in length of these two groups of worms for a given same body weight shows that the general form of the curves are similar, though the field grown worms slightly surpass the length given by the laboratory grown worms (Fig. 4). Generally speaking, however, we may be justified in concluding that the character of growth is essentially the same in these two forms. We further wish to know whether the surface areas of these two forms of earthworm are also similar. In order to calculate the surface of the worms, the following assumption was made. Since the earthworm body is approximately cylindrical in shape, the formula: area of cylinder $= 2\pi rl$ was applied.

In this formula we need to determine the diameter of cylinder or thickness of the body which was found from the following relation;

$$r = \sqrt{\frac{W}{\pi l}}$$

where r is radius, W is weight, l is length.

A preliminary test showed that the actual body weights expressed in grams were found to be approximately equal to the volume of water, expressed in number of c.c., which was displaced when the worms were placed in a narrow cylinder filled with water; for instance, 6 grams in body weight displaced 6 c.c. of water, as will be seen from Table III. From this we may be justified to use the weight (W) instead of volume (v) in the above formula for finding the value of r. As soon as the values of r were found, we can at once compute the body surface from the following formula :

$$S = 2\pi\, rl.$$

TABLE III.

Data of earthworms grown in the field.

Body weight in grs.	Body lenght in mm.	Ratio of body weight and body volume.	Body volume in cc., observed
6.114	186	1.02	6.0
3.213	144	1.19	2.7
1.794	120	1.15	1.5
1.695	120	1.12	1.5
1.164	114	0.97	1.2
0.993	105	1.10	0.9
0.892	100	0.99	0.9
0.759	90	1.08	0.7
0.632	75	1.05	0.6
0.317	69	1.06	0.3
0.313	66	1.04	0.3
0.312	66	1.56	0.2
Average	--	1.11	—

The surface areas computed from the above formula of the forms of earthworms, field and laboratory grown, are given in Table I.

We notice from Fig. 4. that not only the body weights and body lengths show close similarity to each other in the two forms of the earthworms, but that the body surfaces also are similar with respect to corresponding ages or to any given body weights.

With living earthworms a direct determination of body surface is very difficult to perform, owing to the inconstancy of muscular contraction, and therefore a much more uniform value may be better obtained from the formula,

$$S = 2\pi\, rl$$

MORGULIS ('15) has used the formula, $S = k \sqrt[3]{W^2}$, where S is the surface and k a constant factor and W body weight, in order to estimate the surface area in flounders. In that case the value of constant k was found to be 13.44. This value of k in flounders was found to be applicable equally well to estimate the surface area in other higher organisms.

I have also applied the formula, $S = k \sqrt[3]{W^2}$ for calculating the surface of the earthworms, but found that the formula, $S = 2\pi rl$ expresses it more satisfactorily than by using the formula, $S = k \sqrt[3]{W^2}$ for the same purpose.

RESPIRATION.

The quantitative relation which exists between the size of the surface area and an activity of the gaseous metabolism during the growing period of any annelids has not been studied.

For this reason just stated the present research was carried out with a hope to determine the rate of carbon dioxide production with respect to the increasing body surface during growth in the earthworms. That the lower invertebrates, such as the earthworm, which lacks special respiratory organs, may be performing a gaseous change through the external body surface seems quite natural to assume. COMBAULT ('09) presented his view that the external body surface of the earthworm may perform the chief respiratory function and based his hypothesis on the rich supply of blood vessels.

There are several other more or less isolated observations on the carbon dioxide production in earthworms, but I regret to say that most of these data are too inadequate, and further additional data are urgently needed.

The apparatus used to determine the carbon dioxide is one which was devised and described by OSTERHOUT ('18). It may be briefly stated that the apparatus consists of a closed system of glass tubes in which air is circulated by means of a pump.

In the present experiment the closed chamber of 120 cc. capacity was filled with 5 cc. of aqueous solution of Brom thymol blue as the reagent, which was of course renewed in each experiment.

A day-light lamp and white background were used in comparing

the unknown with the known standard indicators, which were prepared according to the method of CLARK. The usual precautions, such as correction of temperature and barometric pressure, and standardizing of indicators by means of a potentiometer, were duly observed.
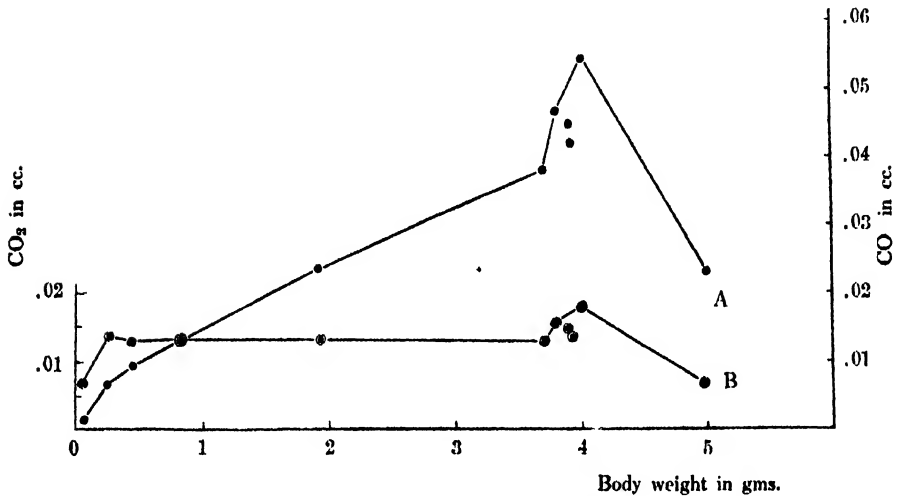

Fig. 5. Showing the rate of carbon dioxide production in association with the increase of body weight.

A. The rate of carbon dioxide production per individual per hour.

B. The rate of carbon dioxide production per sq. cm. per hour.

Observations from the worms having lighter body weight for age are plotted separately in order to indicate that the carbon dioxide production is more closely related with the body weight than with the age.

TABLE   IV.

Relation between the quantity of carbon dioxide and corresponding pH values.

Difference of pH values.	7.0–0.8 0.2	0.3	7.0–6.6 0.4	0.5	7.0–6.4 0.6	0.7	7.0–6.2 0.8	0.9	7.0–6.0 1.0	1.1
CO_2 per cent vol. in cc.	0.018	0.028	0.040	0.054	0.071	0.091	0.114	0.139	0.167	0.197
CO_2 per 5 cc. w. in cc.	0.0009	0.0014	0.0020	0.0027	0.0036	0.0045	0.0057	0.0069	0.0084	0.0099

An attempt was made also to determine the quantity of carbon dioxide corresponding to any pH values by variously diluting the saturated solution of carbon dioxide which was prepared in the generator.

The relation between carbon dioxide content and pH of color indicators, from which intermediate values were read off by interpolation, is shown in Table IV.

Carbon dioxide production in earthworm.

The rate of carbon dioxide production at different ages is shown in Table V. The absolute quantity of carbon dioxide eliminated per individual increases regularly and steadily with increasing age up to the time the clitellum appears, afterwards suddenly increasing, which however is soon followed by a steady decrease, as will be seen from Fig. 5.

TABLE V.

Days.	Average weight in grs.	Average length in mm.	$(S - 2 : rl)$ Body surface computed in sq. cm.	Average CO_2 per individual per hour in cc.	Average CO_2 per sq. cm. per hour in cc.	Average CO_2 per kilo per hour in cc.
(IV. 3. cocoon)	0.0204			0.00011		
14	0.048	40	1.58	0.00102	0.0065	20.9
28	0.258	49	4.72	0.00690	0.0146	26.7
42	0.419	80	6.48	0.00890	0.0137	23.0
56	0.822	106	10.45	0.01370	0.0130	16.6
70	1.944	146	18.88	0.02440	0.0129	12.6
98 (mature)	3.775	182	29.37	0.03790	0.0129	10.1
108	3.826	182	29.60	0.03720	0.0159	12.5
116	3.996	182	30.55	0.05450	0.0178	13.6
128	3.926	185	30.21	0.04500	0.0149	11.5
134	3.918	185	30.21	0.04200	0.0139	10.7
242	5.050	185	34.27	0.02420	0.0071	4.8

The rate of carbon dioxide production per unit of surface area.

The relations of carbon dioxide production per unit of surface area are nearly constant during the growing period up to when the clitellum appear, while during the first two weeks after hatching it shows a steady increase.

As soon as the clitellum appears and the reproductive system begins

to show greater development, the carbon dioxide production per unit area increases suddenly, which in turn decreases again till the end of the observation.

BOUNHIOL ('02) found a similar relation with the 20 species of polychaeta in which the individuals with lighter body weight give a greater amount of carbon dioxide per unit weight than the individuals with heavier body weight. However, the earthworms differ from the polychaeta in one respect; that is, carbon dioxide production increases after the worms reach maturity, instead of showing a continuous decrease with increasing body weight, as was the case with younger actively growing earthworms.
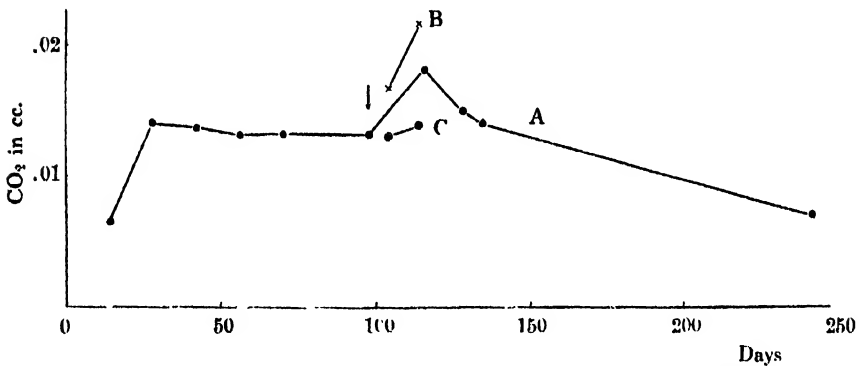
Fig. 6. Showing the rate of carbon dioxide production per sq. cm per hour. in the anterior and in the posterior portions of the earthworm.
A. Whole body.   B. Anterior portion.   C. Posterior portion.

In oder to test whether or not the rapid development of all the sexual organs is responsible for the sudden increase of carbon dioxide after the appearance of the clitellum, the anterior portion of the worm, which contains practically all the reproductive organs, was compared with the posterior portion, which is devoid of those organs. It was found that the quantity of carbon dioxide given off by the anterior segments is far greater than that given by the posterior portion; while the posterior portion gives a similar amount of carbon dioxide to that given by the whole immature worm.

This seems to indicate that the rapid increase of carbon dioxide which was mentioned above is related to the development of sexual organs.

This statement seems further supported by the observation made on the anterior segments of the two earthworms, in one of which the clitellum has just appeared, while in the other nearly two weeks has elapsed since its appearance. It was found that the former gives off a less amount of carbon dioxide than the latter, suggesting that the greater the development of the sexual organs the greater is its production of carbon dioxide; while the posterior segments of these two worms gave approximately the same amount of carbon dioxide as the whole immature worm. Table VI, and Fig. 6 illustrate the relations just mentioned.

TABLE   VI.

No. of observ.	Parts.	Segments.	Weights in grs.	Surface computed in sq. cm.	CO_2 out put per individual per hour in cc.	CO_2 but put per sq. cm. per hour in cc.
1	ant.	39	1.701	14.1	0.027	0.019
	post.	62	1.951	15.6	0.020	0.013
2	ant.	37	1.462	12.9	0.027	0.021
	post.	66	1.278	11.8	0.014	0.012
3	ant.	33	1.268	11.7	0.027	0.023
	post.	73	1.818	14.9	0.020	0.0136
4	ant.	34	1.544	13.3	0.031	0.024
	post.	49	1.983	15.7	0.027	0.017
Average	ant.	36	1.494	13.0	0.028	**0.0216**
	post.	63	1.758	14.5	0.020	**0.0138**
(Observation on 27th of July at 25°C.)						
1	ant.	28	1.293	11.8	0.020	0.017
	post.	73	1.858	15.1	0.020	0.013
2	ant.	37	1.688	14.2	0.023	0.016
	post.	64	2.143	16.6	0.020	0.012
Average	ant.	33	1.491	13.0	0.022	**0.0167**
	post.	69	2.000	15.9	0.020	**0.0128**
(Observation on 8th of August at 24°C.)						

The respiration of annelids has been studied by many workers; COMBAULT ('09) considers that the skin of earthworms is the important organ of respiration, based on the fact that the epidermal layer is especially rich in blood capillaries; WILLIAMS found that the mucus

membrane of earthworms has great ability to absorb oxygen; BRANDES ('07) and STEPHENSON ('13) consider that the wall of the intestine of earthworms, especially of polychaetous annelids, can also take part in respiration. BOUNHIOL ('02) found that in polychaetous annelids the rate of carbon dioxide production was greater in the smaller individuals, as was noted in many other animals; as for instance by CHILD ('19) for the oxygen consumption of planaria, ALLEN ('19) for the carbon dioxide of planaria, VERNON ('95) for the oxygen consumption of hydromedusae.

RUBNER ('83) stated that the gaseous metabolism is proportional to the body surface and that per unit of area it is the same for all vertebrates.

The theoretical basis for this generalization is the simple physical principle of radiation of heat which is proportional to the exposed surface.

Since the metabolic processes are associated with the production of heat, the latter depending largely upon the rate of its dissipation to the outside environment, the metabolic processes must bear a direct relation to the surface. PÜTTER stated against RUBNER's theory that the body surface has no relation to metabolic processes but that it is related to the area of the organs or tissues which are directly connected with the gaseous metabolism. MORGULIS ('15) found that, while per unit of body weight oxygen consumption diminishes as the size of the flounder increases, the oxygen consumption per unit of body surface increases very regularly. In other words, the larger the flounder the larger its oxygen requirements per square cm. This obviously results from the direct dependence of the metabolic exchange upon the mass of the organism which grows more rapidly than the surface. In the Japanese earthworm, as has been shown in Fig. 7, the carbon dioxide production is essentially proportional to the body surface as RUBNER stated. This very obviously results from the fact that the function of respiration is chiefly performed by the skin and it is natural then that the increase of carbon dioxide production must be associated with the body surface, differing from the flounder above mentioned or from other higher organisms which are provided with special respiratory organs.

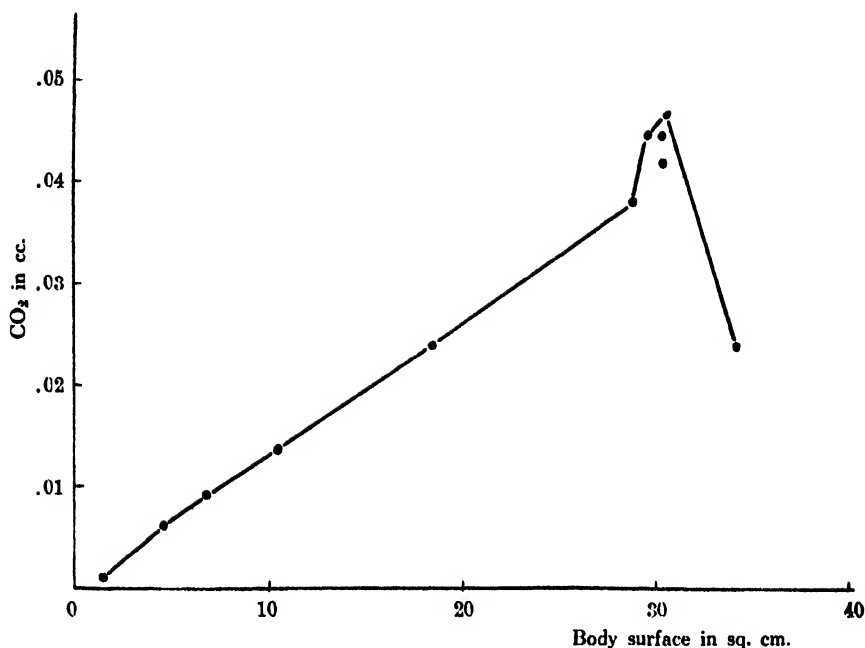In the very early stage of life the rapid increase of the rate of

Fig. 7. Showing the rate of carbon dioxide production per individual per hour in association with the increase of body surface.

Observation from the worms having smaller body surface for age are plotted separately in oder to indicate that the carbon dioxide production is more closely related with the body surface than with the age.

carbon dioxide production which was noted was due to the fact that the development of capillaries during this early period soon after hatching was not yet completed, thus giving relatively a lesser degree of gaseous exchange. As soon as the vascularisation in the epidermal layer is completed the carbon dioxide production becomes the function of the area of the body surface, or in other words, the rate of carbon dioxide production per hour becomes constant, irrespective of the size of the earthworm. When, however, the sexual organs begin to develop rapidly, the rate of carbon dioxide production rises in conformity with the increase of metabolic rate.

After passing through the active breeding period, all the sexual organs including all other tissues appear to enter the retrogressive state.

The rate of metabolism under such conditions would gradually diminish and give off a less amount of carbon dioxide per individual or per surface area than was actually found.

SUMMARY.

1. In *Pheretima communissima*, GOTO et HATAI, which was hatched and raised in the laboratory, the clitellum appears about in 16 weeks; while in those grown in a field it appears in 14 weeks.

2. After the appearance of the clitellum the growth of the body length practically ceases but the body weight continues to grow, though slightly.

3. In conformity with the other higher animals the growth rate decreases with increasing age, but this relation is much disturbed with the sudden increase of the sexual organs and products in the adult stage.

4. The body surface of the earthworms may be estimated satisfactorily by the formula $S = 2\pi rl$, and also the formula $S = K\sqrt[3]{W^2}$, may be used, but its results are not as satisfactory as those of the former.

5. The gaseous metabolism of the the earthworm bears a direct relation to the body surface, and the carbon dioxide production per sq. cm. per hour is constant during the greater part of the growing period.

6. When the sexual organs begin to develop rapidly the rate of carbon dioxide production per sq. cm. per hour rises rapidly, but after cessation of the breeding period it again falls off steadily, parallel with the advance of the retrogressive process of these organs.

LITERATURE.

ALLEN, G. D. 1919. Quantitative studies on the rate of respiratory metabolism in planaria. II. The rate of oxygen consumption during starvation, growth and regeneration in relation to method of susceptibility to potassium cyanide as a measure of rate of metabolism. Amer. Journ. Physiol., Vol. 49. p. 420.

BARCROFT. J, 1925. Respiratory function of blood London. pp. 63-74.

BAYLISS, W. M. 1924. Principles of general Physiology.

BOUNHIOL, J. 1902. Recherches biologiques sur la respiration des annélides polychètes. Ann. d. Sc. natur., 16.

BRODY, S. 1926. Time relation of growth. I. Genetic growth constants of animals. Journ. Gen. Physiol., Vol. 8. p. 233.

BRODY, S. 1928. A comparison of growth curves of man and other animals. Science. Vol. 67. pp. 43–6.

CALDWELL, G. T. 1925. A reconnaisance of the relation between desication and carbon dioxide production in animals. Biol. Bull. Vol. 48. p. 259.

CHILD, C. M. 1919. A comparative study of carbon dioxide production during starvation in planaria. Amer. Journ. Physiol., Vol. 48. p. 231.

COMBAULT, T. 1909. Contribution à l'étude de la respiration et de la circulation des *Lumbricus*. Journ. Anat Physiol., Vol. 45. p. 358.

DUBOIS, D. and E. F. 1916. A formula to estimate the approximate surface if weight and height are known. Arch. Internal. Med., Vol. 17.

HALDANE, J. S. 1921. Respiration. London.

HYMAN. L. H. 1918. Carbon dioxide production in relation to regeneration in planaria. Amer. Journ. Physiol., Vol. 48. p. 340.

HYMAN, L. H. 1919. The rate of respiratory metabolism in planaria. Amer. Journ. Physiol., Vol. 49. p. 420.

HYMAN, L. H. 1919. Physiological studies on planaria. III. Oxygen consumption in relation to age (size) differences. Biol. Bull., Vol. 37. p. 388.

KLEINENBERG, N. 1879. The development of earthworm, *Lumbricus Trapezoides*, DUGÈS. Quart. Journ. Micro. Sci., Vol. 19. pp. 206–244.

KROGH, A. 1916. The respiratory exchange of animals and men. London.

LEE, MINOT, O. 1925. On the mechanism of respiration in certain orthoptera. Journ. Exp. Zool., Vol. 41. No. 2, pp. 125–154.

MORGULIS, S. 1915. The body surface of flounders and its relation to the gaseous metabolism. Amer. Journ. Physiol., Vol. 36. p. 207.

OSTERHOUT, W. J. V. 1918. A method of studying respiration. Journ. Gen. Physiol., Vol. 1. p. 17.

OSTERHOUT, W. J. V. 1919. Comparative studies on respiration. Journ. Gen. Physiol., Vol. 1. pp. 171–179.

PÜTTER, A. 1911. Active Oberfläche und Organfunktion. Zeitsch. f. Allgem. Physiol., Bd. 12. S. 133.

ROBERTSON, T. B. 1923. The chemical basis of growth and senescence. Philadelphia and London.

ROGERS, C. G. 1927. Textbook of comparative physiology. New York. p. 239.

SCHMIDT, P. 1918. Anabiosis of the earthworm. Journ. Exp. Zool., Vol. 27. p. 57.

STEPHENSON, J. 1913. On intestinal respiration in annelids: with considerations on the origin and evolution of the vascular system in that group. Trans. R. Soc. Edinburgh., Vol. 49. p. 735.

THOMPSON, D'ARCY, W. 1916. On growth and form. Cambridge University Press.

VERNON, H. M. 1895. The respiratory exchange of the lower invertebrates. Journ. Physiol., Vol. 19. p. 18.

WINTERSTEIN, H. 1921. Handbuch der vergleichenden Physiologie. Bd. 1.

YAGI, M. 1926. Analysis of the growth curves of the insect larvae. Mem. Coll. Agri. Kyoto. Imp. Univ., No. 1.

Physiological Studies on the Eel.
I. The Seasonal Variation of the Blood Constituents.

By

NOBUYUKI KAWAMOTO.

(From the Physiological Laboratory of the Komine Institute,
Nishigahara, Takinogawa, Tokyo, Japan).

INTRODUCTION.

PEARCE ('25) stated that the chemical composition of certain fishes changed according to season.

HALL, GRAY and LEPKOVISKY ('26) found that the blood constituents of fishes show some alteration by asphyxation, and SCHAEFER ('27) reported the decrease in the number of blood corpuscles in fishes during winter.

From these variations, above mentioned, in the bodily compositions we might anticipate a greater or less degree of alteration in the blood gases in fishes influenced by the seasons, but so far as I am aware no investigators except perhaps HALL ('28) paid much attention to this point.

The present work was therefore undertaken to determine whether or not our anticipation as to the influence of seasonal variations to the blood constituents of eel would be realized.

For this purpose a monthly determination was made, in the Physiological Laboratory of the Komine Institute, on the number and relative volume of corpuscles, viscosity, and content of iron and non-protein nitrogen in the blood.

MATERIALS AND METHODS.

The eels (*Anguilla japonica* T. & S.) employed in this experiment were obtained always from the fish cultural pond at Kawasaki, Kana-gawa Prefecture, and the fishes were used within two hours after being brought into the laboratory.

The blood was quickly collected from the heart by means of a hypodermic syringe by fixing the fish on a wooden plate. The blood was mixed with potassium oxalate in a dish to avoid both coagulation and evaporation.

These experiments were extended from July, 1928 to June, 1929.

Viscosity ...Viscosity was determined by the HESS's viscosimeter immediately after the blood was collected for it shows alteration quickly, as LECOMTE DU NOÜY has noted ('28).

Corpuscles....The number of red corpuscles per c.mm. was enumerated with THOMA-ZEISS's haemocytometer by diluting the blood with 0.85 per cent. salt solution.

Relative volume of corpuscles....The relative volume of corpuscles and plasma were determined by means of the haematocrite which was revolved for forty minutes at the rate of about 3,500 r. p. m.

Non-protein nitrogen....mg. of non-protein nitrogen per 100 cc. of the blood was determined by the BANG's method ('20) by using 0.25 cc. in each test.

Iron.. mg. of Fe per 100 cc. of the blood was determined by PINCUSSEN method ('28) from 0.2 cc. blood in each test.

All the above determinations were carried out on the eels of approximately the same body weight of about 150 gms.

To determine whether or not the number of red corpuscles regularly varies according to the size, I examined eels with body-weights from 0.78 to 170.00 gms. at the Toyohashi Fish Cultural Station of Imperial Fisheries Institute in the middle of July, 1929.

RESULTS.

Five eels of approximately the same weight were examined each month.

Average of five analyses taken from each eel is given in Table I.

TABLE I.

Average results of blood constituents examined monthly.

Date	Body weight gm.	Body length mm.	No. corpuscles in 1 cmm.	Relative Volume %	Viscosity*	Fe-content mg in 100 cc.	Non-protein N mg in 100 cc.
July 9.	227.5	695	2,080,000	27.0	—	31.1	57.7
Aug. 1.	105.0	427	2,901,000	31.0	3.50	28.2	125.6
Sept. 10.	120.0	440	2,880,000	27.5	3.45	29.5	78.0
Oct. 10.	142.5	448	2,461,000	26.7	3.18	26.0	62.9
Nov. 20.	155.0	455	2,175,000	27.2	2.98	25.8	41.0
Dec. 11.	193.0	486	2,960,000	28.8	3.40	37.1	35.8
Jan. 16.	138.0	429	2,570,000	33.5	3.57	32.6	34.0
Feb. 14.	174.0	495	2,490,000	29.7	3.20	30.2	26.3
Mar. 3.	201.0	536	2,210,000	24.0	2.97	31.4	38.8
April 9.	117.5	428	2,030,000	25.0	3.27	31.5	46.0
May 14.	128.0	440	2,180,000	25.9	3.33	33.9	35.7
June 17.	184.0	485	2,370,000	27.4	3.36	32.4	44.6

* temperatures between 14—18°C.

The temperatures of pond water and of the atmosphere were taken at noon daily except in the winter and these are shown in Table II, and graphic presentation in Figs. 1 and 2.

TABLE II.

Average temperatures of the pond water and atmosphere.

Date	Temperatures	
	Atmosphere	Water
1928	°C	°C
July	25.0	25.3
Aug.	26.8	26.9
Sep.	27.4	27.2
Oct.	20.2	19.1
Nov.	15.9	13.2
Dec.	9.2	6.7
1929		
Jan. 16.	14.5*	7.5*
Feb. 14.	7.3*	5.0*
Mar. 3.	13.5*	12.5*
April	18.3	15.6
May	15.7	18.7
June	24.2	23.5

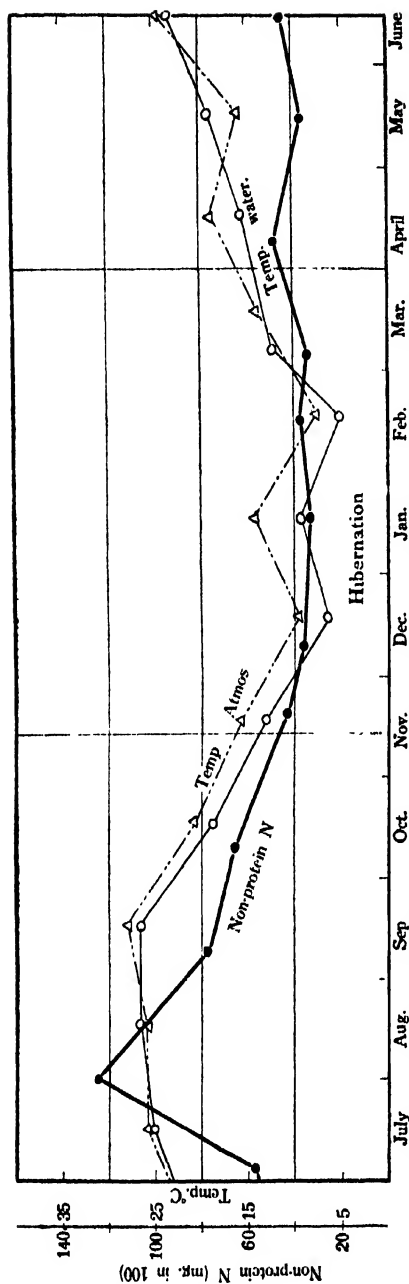* temperatures at the day when eels were examined.

Fig. 1. Showing the relation between the non-protein nitrogen in the blood and the temperatures of atmosphere and water.
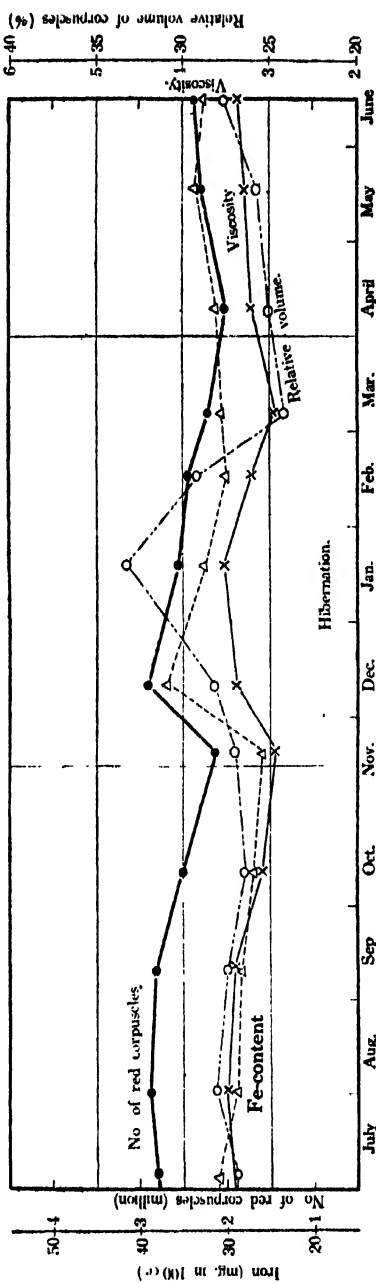

Fig. 2. Showing the seasonal variation of relative volume, number of corpuscles, viscosity and iron content.

In Table III are shown the relation between the number of red corpuscles and weights of the eels, dividing them into smaller, medium, and larger groups.   The enumeration of red corpuscles was made soon after the eels were captured from the pond.

TABLE III.

Relation between the number of red corpuscles and measurements of eels.

Group	Body length mm.	Body weight gm.	No. of red corpuscles in 1 cmm.
smaller	98	0.78	2,768,000
	103	1.09	2,984,000
	105	0.79	3,000,000
	106	1.12	2,804,000
	111	1.25	2,648,000
	Average ························· 2,855,000		
medium	263	22.00	2,304,000
	321	30.50	2,404,000
	346	90.50	2,570,000
	390	104.50	2,528,000
	376	87.50	3,128,000
	Average ························· 2,572,100		
larger	412	93.00	2,648,000
	412	105.00	2,520,000
	426	170.00	2,612,000
	435	161.00	2,224,000
	441	152.00	2,712,000
	Average ························· 2,546,000		

The data given above show clearly the seasonal variations in blood constituents of eels, as will also be seen from Figs. 1 and 2.

The variations of blood constituents indicate the two types, (1) the non-protein nitrogen increases in summer and decreases in winter coinciding approximately with respective rise and fall of temperature of water and atmosphere (see Fig. 1).   On the other hand (2) the number, relative volume, viscosity and iron content of the red blood corpuscles show conspicuous increase as soon as hibernation begins and decrease as it approches its end (see Fig. 2).

DISCUSSION.

Table III shows that the number of red corpuscles presents little

variation in all the specimens examined, despite the fact of greater differences in body weights; and on the other hand the eel blood indicates true variation according to the seasons. Since the variation arising from the body sizes are insignificant, we may be able to eliminate the individual variations if the eels of approximately the same weights were used.

AUDIGÉ ('21) reported in his paper that the growth rate of fishes runs parallel with water temperature, and I have also noted an increase of non-protein nitrogen which runs parallel with the rise of temperatures (Fig. 1).

From these coincidences between the body growth on one hand and increase of non-protein nitrotein on the other, it seems as though the increase of the latter in the blood of eel can be taken as an index of increasing metabolic activity.

This assumption seems further supported by the fact that the non-protein nitrogen is minimum and is almost constant in winter, where the metabolic activity is almost at a standstill due to the natural starvation resulting from hibernation which last from the middle of November to the end of March of the next year, during which period the growth rate is least.

SCHAEFER ('26) reported in his paper that the number of red corpuscles in " pumpkinseed ", *Eupomotis gibbosus* L. decreased during winter but returned to normal in spring, and in my own present experiment similar phenomenon is observed (see Table I, column 4) through it shows slight increase in the beginning of hibernation. However this increase in the number of red corpuscles at the beinning of hibernation seems to indicate that the water of the blood is perphaps absorbed into the body tissue, thus resulting in apparent increase of corpuscles.

During fasting the tissue becomes acidic and absorbs more water, in other words the loss of water gives rise to greater concentration of the blood which in turn would produce greater concentration of blood corpuscles in the given same volume.

This abnormal absorption of water during fasting must be of a reversible character since the number of red corpuscles becomes less again as soon as the hibernation or fasting period comes to an end.

Relative volume of corpuscles and iron content runs parallel with

the change of the number of red corpuscles, and the viscosity as MATHEWS ('24) stated, is also a function of the number of corpuscles, all of which are seen clearly from my own present work (Table I, and Fig. 2).

SUMMARY.

1. The seasonal variation is indicated by the changes in constituents of the blood of eels.
2. Non-protein nitrogen in the blood may be taken as an index of metabolic activity.
3. Relative volume, number of corpuscles, viscosity and iron content gradually decrease during winter till these begin to rise considerably in the beginning of hibernation but decrease again in the spring.
5. It was suggested from the present study that such seasonal variation of the blood constituents may be partly responsible for the variations of blood gases noted by other investigators.

BIBLIOGRAPHY.

1) AUDIGÉ, P.* 1921. Comp. rend. soc. biol., LXXXIV, 2.
2) BANG, I. 1921. Mikrobestimmung zur Blutuntersuchung. Wiesbaden. 23.
3) HALL, F. G., I. E. GRAY and S. LEPKOVSKY, 1926. J. Biol. Chem. LXII, 549-554.
4) HALL, F. G. 1928. J. Biol. Chem. LXXVI, 623-631.
5) LECOMTE DU NOÜY. 1928. Science, LXVII, 563-564.
6) MATHEWS, A. P. 1924. Physiological Chemistry. London. 512.
7) PEARCE, A. S. 1925. Ecology, VI, 7-16.
8) PINCUSSEN, L. 1928. Mikromethodik Quantitative Bestimmung der Harn-, Blut- und Organbestandteile in kleinen Mengen. Leipzig. 99-100.
9) SCHAEFFR, A. A. 1926. J. Gen. Physiol., VII, 3, 341-343.

    * cited indirectly.

Physiological Studies on the Eel.

II. The Influence of Temperature and of the Relative Volumes of the Red Corpuscles and Plasma upon the Haemoglobin Dissociation Curve.

By

NOBUYUKI KAWAMOTO.

(From the Physiological Laboratory of the Komine Institute,
Nishigahara, Takinogawa, Tokyo, Japan).

CONTENTS.

The gases of the blood of fishes have been studied by several authors from various standpoints, especially by JOLYET and REGNARD ('77), KROGH and LEITCH ('19) and WASTL ('28). KROGH and LEITCH ('19) studied the influence of the temperature upon the respiratory function of the blood of fishes.

In the present investigation I dealt more specially with the haemoglobin dissociation curve of the blood, which is evidently influenced by the variations of its constituents as well as by the variation of temperature, as has been already suggested in my former paper (KAWAMOTO '29).

MATERIALS USED AND TECHNIQUE EMPLOYED.

The material used in my experiment was *Anguilla japonica* TEM-

MINCK & SCHLEGEL, a common eel living in the fresh and brackish waters all over Japan excepting in the Northern part of the Japan Sea (MARUKAWA '16).

The eels were purchased at market and kept alive in an aquarium in the laboratory without feeding, often for about two months.

The eels used measured from 445 to 790 mm. in length and from 163 to 880 gms. in weight.

The sexes were not recorded as their difference probably does not influence the gases of blood, for I have never noted such influence in many kinds of fish as far as I have tested.

The blood was collected by the injection apparatus directly from the ventricle of the heart after opening the perivisceral cavity.

Neither narcotic nor anticoagulant was used, as these might have influenced the normal constituents of the blood. However, for the determination of hydrogen ion concentration of the blood, 0.3 per cent. potassium oxalate was used to prevent coagulation.

The determination of the oxygen capacity of fish blood is very difficult, and the ordinary ferricyanide method (HALDANE '22) can not be used at all, since a fibroid clot will always be formed after the blood is laked, on adding ferricyanide.   .

KROGH and LEITCH ('19) and WASTL ('28) also report such phenomenon on the fish blood, and MACELA and SELISKAR ('25) on the frog blood. For this reason, instead of the usual manometric method, I have used the "constant volume" apparatus of VAN SLYKE and NEIL ('24 and '27).

For general technique employed in the present investigation, the reader is referred to my former paper (KAWAMOTO '28).

The gas chain method was used for determining the hydrogen ion concentration of the blood which was kept under the paraffin oil.

FREISCHL-MIECHER haemoglobinometer (DOMARUS '21) was found unfitted for determining the haemoglobin contents, for, I found that a fibrin like clot was formed after complete haemolysis by water or by 1‰ Na_2CO_3 solution, which disturbs the uniform distribution of color, though both reagents are usually used with satisfaction in the blood of higher mammals. I have therefore determined the relative volume of the red corpuscles by a haematocrite, by revolving it for 40 minutes with a speed of about 3,500 revolutions p. m.

EXPERIMENTS.

1. On the hydrogen ion concentration of the blood during starvation.
Fifteen eels were caught with a fish net in the fish cultural pond at Kawasaki near Tokyo and brought to the laboratory. After these were left quietly for about two hours in the laboratory aquarium, the venous blood was collected from five eels and was oxalated under paraffin oil for the purpose of determining the value of pH; and these data were used as the control. The remaining ten eels were kept in the aquarium without giving any food, and value of pH was determined after fourteen days and after forty-three days respectively.

The data taken from the above tests show apparently no change in the value of pH during at least the period of starvation for about six weeks, with the exception of the eels of fourteen-day starvation series, in which a negligible increase of 0.03 in the value of pH was noted.

TABLE 1.

The pH value of blood (oxalated, under paraffin oil) of eels under starvation.

Date	Starv. days.	pH	Average.
Oct. 15	0	7.60	
,,	,,	7.62	
,,	,,		7.62
,,	,,	7.64	
,,	,,	7.66	
Oct. 29	14	7.62	
,,	,,	7.67	
,,	,,	,,	7.75
,,	,,		
,,	,,	7.70	
Nov. 27	43	7.58	
,,	,,	7.61	
,,	,,	7.62	7.62
,,	,,	7.66	
,,	,,	7.70	

2. On the variation of the relative volume of red corpuscles.
The relative volume of red corpuscles shows considerable variation, ranging from 23.0 to 53.2 per cent.

KROGH and LEITCH ('19) state that " the same (of dissociation

curve of haemoglobin in the carp) holds good also for the blood of
eel, though the result obtained on the different specimens appears to
be rather discordant ", and McCormic and Macleod ('25 from Wastl)
reported that the haemoglobin content changes if fishes are kept
longer in the laboratory aquarium.

TABLE 2.

Frequency distribution of the relative volume of red corpuscles.

Relat. vol. (%)	Frequency	Cumulative freq.
20—25	2	2
25—30	2	4
30—35	8	12
35—40	20	32
40—45	17	49
45—50	4	53
50—55	1	54
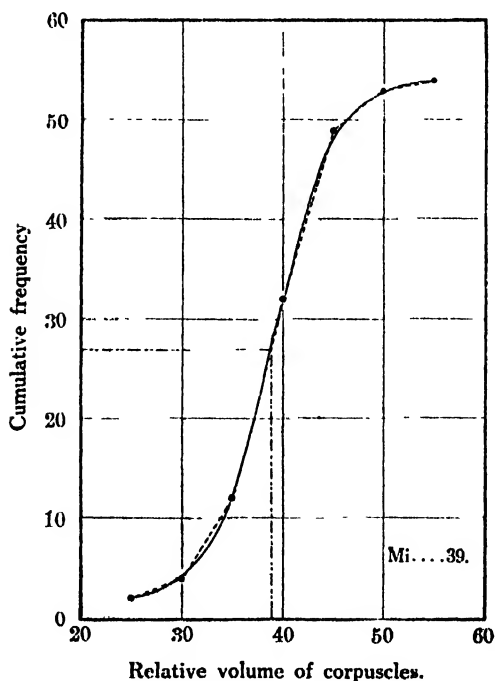
Fig. 1.   Showing the relation between the median value, cumulative
frequency and relative volume of corpuscles.

Since the variation of haemoglobin content as well as of red corpuscles is considerably high, it appears unsafe to compare directly such data, as for instance, the gaseous metabolism which is intimately related with the conditions of the red blood corpuscles, without some knowledge of the relative volume of red corpuscles in the species of fishes under consideration.

In the eel, the median value of the relative volume of red corpuscles, which was evaluated by the method of cumulative frequency (OGURA '25) was found to be 39 per cent. (Table 2, Fig. 1), and is conveniently called " standard value ".

3. *The influence of temperature and of the relative volumes of the red corpuscles and plasma upon the oxygen capacity of the blood of eel.* The oxygen capacities of the blood were determined, which show

TABLE 3.

The relation between the oxygen capacity and the relative volume
of corpuscles (at 17'$\pm$0.3 C).

Relative volume of corpuscles (x)	O$_2$-capacity vol. % (y)	corresponding O$_2$-capacity at 39 vols. % corpuscles	Deviation
31.0	10.2	12.84	−0.07
,,	10.4	13.10	−0.41
,,	,,	,,	,,
31.2	10.8	13.50	−0.01
31.5	10.9	,,	,,
32.0	11.0	13.42	−0.09
,,	11.2	13.66	+0.15
,,	11.3	13.80	+0.31
34.0	11.7	13.98	−0.13
36.0	12.0	13.00	−0.51
36.5	12.5	13.36	−0.15
,,	12.6	13.46	−0.05
37.0	12.8	13.50	−0.01
,,	12.9	13.60	+0.09
,,	13.5	14.22	+0.71
39.0	,,	13.50	−0.01
,,	13.6	13.60	+0.09
39.5	,,	13.43	−0.08
40.0	13.7	13.37	−0.14
,,	14.0	13.66	+0.15
,,	,,	,,	,,
,,	14.1	13.76	+0.25
,,	14.3	14.96	+0.45
41.0	14.5	13.80	+0.29
,,	14.6	13.89	+0.38
Average 13.51			

different relative volumes, at the temperatures of 5°, 17° and 30°C respectively, using the air from which carbon dioxide was removed. The results are given in Tables 3, 4 and 5, and in Fig. 2.

TABLE 4.

The relation between the oxygen capacity and the relative volume of corpuscles (at 5°±0.3C).

Relative volume of corpuscles (x)	O_2-capacity vol. % (y)	Corresponding O_2-capacity at 39 vols. % corpuscles	Deviation.
29.5	11.2	14.81	+0.16
31.0	11.5	14.49	—0.16
34.0	12.6	14.46	—0.19
35.0	13.3	14.75	+0.10
37.0	14.0	14.76	+0.11
38.0	14.1	14.49	—0.16
40.5	15.0	14.45	—0.20
"	15.5	14.62	—0.03
41.5	15.7	14.77	+0.12
43.0	16.5	14.96	+0.31
45.0	17.0	14.73	+0.08
46.5	17.6	14.75	+0.10
"	17.7	14.84	+0.19
Average		14.65	

TABLE 5.

The relation between the oxygen capacity and the relative volume of red corpuscles (at 30°±0.5°C).

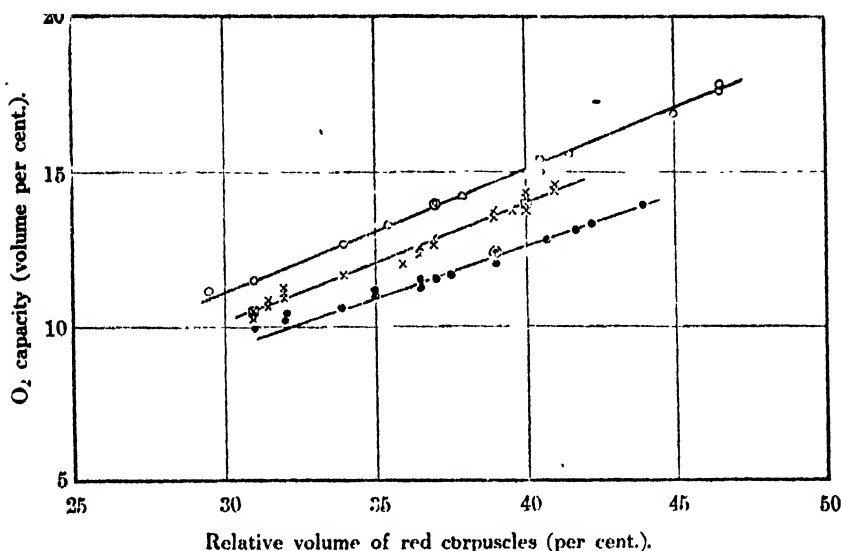| Relative volume of corpuscles (x)	O_2-capacity vol. % (y)	Corresponding O_2-capacity at 39 vols. % corpuscles	Deviation.
31.0	10.0	12.59	+0.27
32.0	10.2	12.42	+0.10
"	10.3	12.55	+0.23
34.0	10.5	12.44	+0.12
35.0	11.0	12.26	—0.06
"	11.2	12.49	+0.17
36.5	11.1	11.86	—0.46
"	11.4	12.19	—0.13
37.0	11.6	12.21	—0.11
37.5	11.5	11.99	—0.33
39.0	12.0	12.00	—0.32
"	12.6	12.60	+0.28
40.7	12.8	12.25	—0.07
41.5	13.0	12.22	—0.10
42.0	13.2	12.25	—0.07
44.0	14.0	12.41	+0.09
Average		12.32	

Fig. 2. Showing relation between oxygen capacity and relative volume of corpuscles, o... at 5°C, ×....at 17°C, ●.. at 30°C, large circle around the above three marks shows that the values of two observation are indicated.

Taking the relative volume of red corpuscles (*x*) as abscissa, and the percentage saturation of the oxygen capacity (*y*) as ordinate, the relation between these, at 5°, 17° and 30° respectively, can be represented by a linear equation in the range between 23 to 53 per cent. of the relative volume of red corpuscles :

$$y = ax + b \ ... (1)$$

The value of the two constants *a* and *b* at different temperatures are given below :

Temp.°C.	a	b
5	0.419	−1.663
17	0.407	−2.176
30	0.386	−2.668

As will be seen in Fig. 2, the observation points are nearly evenly distributed on both sides of the straight lines from the formula, showing that the relation between oxygen capacity and relative volume of corpuscles can best be represented by the linear equation.

4. *The relation between the haemoglobin dissociation curve and the influence of temperature.*

The defibrinated blood obtained from each specimen was saturated

TABLE 6.

(30±0.5°C).

O_2-tension mm.	O_2-capacity vol. %	Corpuscles, Relative vol. %	Corresponding O_2-capacity at 39 vol. %
2.0	4.5	38.0	4.6
,,	,,	,,	,,
,,	5.0	37.5	5.2
6.9	8.5	42.0	7.8
,,	8.4	,,	,,
,,	8.5	,,	,,
14.5	10.5	39.0	10.5
24.4	12.9	45.5	10.8
,,	13.0	44.0	11.5
38.0	11.2	37.0	11.8
,,	11.5	37.5	11.9
50.0	12.3	40.0	12.0
70.0	12.6	39.0	12.6
122.0	11.7	37.5	12.1
160.0	11.8	36.5	12.6
,,	11.1	,,	11.8
,,	14.1	44.0	12.4

TABLE 7.

(17±0.3°C).

O_2-tension mm.	O_2-capacity vol. %	Corpuscles, Relative vol. %	Corresponding O_2-capacity at 39 vol. %
4.0	6.8	32.0	8.0
,,	,,	,,	,,
5.6	10.1	40.0	9.8
,,	9.6	,,	9.3
11.8	11.9	37.8	12.2
24.1	13.6	40.0	13.2
,,	13.3	,,	12.9
36.0	13.5	39.0	13.9
,,	12.9	,,	12.9
,,	14.0	40.0	13.6
41.8	13.7	,,	13.3
,,	10.9	31.2	13.6
70.0	13.3	39.5	13.3
160.0	11.2	32.0	,,
,,	11.3	,,	13.7
,,	11.0	,,	13.4
,,	14.3	40.0	13.9
,,	13.5	,,	13.1

with the mixed gas having various degrees of oxygen tension, and the oxygen capacities were estimated at the temperatures at 5°, 17° and 30°C. The results are shown in Tables 6, 7 and 8.

TABLE 8.

(5±0.3°C).

O -tension mm.	O₂-capacity vol. %	Relative vol. blood %	Corresponding O₂-capacity at 59 vol. %
3.9	11.5	41.5	10.8
5.4	15.4	48.0	12.5
,,	12.6	39.5	12.4
17.3	17.8	46.5	14.9
,,	17.0	,,	14.2
25.4	11.0	29.5	14.5
28.3	15.0	40.5	14.4
36.5	13.0	35.0	14.5
55.0	13.7	37.5	14.3
97.0	,,	41.0	13.2
,,	14.6	39.0	14.0
160.0	15.7	41.5	14.7
,,	14.0	38.0	14.4

The values given in column 2 of these tables showing the relation between the oxygen capacities and the increase of the oxygen tension are highly irregular, caused by relatively higher variations of the
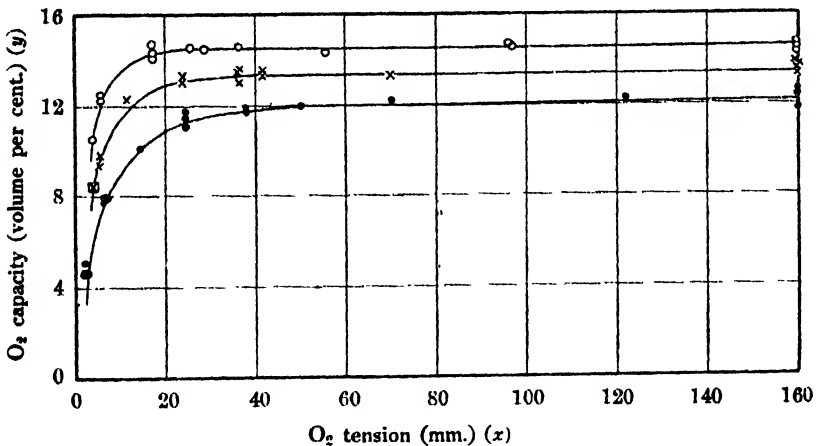
Fig. 3. Showing the curves of haemoglobin dissociation at varied temperatures with oxygen capacity in volume per cent. O··· 5°C, ×··· 17°C, ●···· 30°C, large circle around above marks shows the points that doubled.

relative volume of corpuscles in each blood sample. However, these irregularies are minimized when these are reduced proportionately to 39 per cent. or "standard value" and I have conveniently utilized these reduced figures for various later calculations.

The data given in Tables 6, 7 and 8 are shown graphically by the three curves in Fig. 3. It will be noted from these curves that less oxygen is taken up by the blood when the temperature becomes higher as is generally the case with the blood of other animals (BARCROFT '14, MACELA and SELISKAR '19).

The Oxygen capacity increases rapidly till the oxygen tension reaches 10 mm. Hg. but it reaches almost a standstill at 20 mm. Hg. in the case of 5°C, while at 17°C the former continues to increase up to 25 mm. Hg., though it increases very rapidly up to 8 mm. Hg.; and finally at 30°C it increases upon to 45 mm. Hg..

The final values of O_2 capacity corresponding to the oxygen tension of 160 mm. Hg. given at 5°, 17°, and 30°C are 14.50, 13.50 and 12.25 vols. per cent. respectively.

These final values of the O_2 capacities correspond with the ordinate values in Fig. 2 which correspond to the standard value of the relative volume, or 39 per cent., in the abscissa in the following:

Temp.°C	From dissociation curve.	From table 3, 4 and 5.
5	14.5 vols. %	14.65 vols. %
17	13.50	13.51
30	12.25	12.32

As will be seen from this table, that differences between the values of O_2-capacity derived from the curves and those actually found are very slight, indicating the accuracies of the technique employed here.

GENERAL CONSIDERATION

As will be seen from the data already given, the difference of the relative volumes of red corpuscles varies considerably according to the physiological state of the individuals, and I now wish to consider various factors which appear to me to cause such differences.

It was found that the values of oxygen capacity at 160 mm. of oxygen tension were 14.5 vols. per cent. at 5°C, 13.5 vols. per cent. at 17°C and 12.2 vols. per cent. at 30°C respectively (Tables 6-8).

Since these values are directly comparable with the values obtained in using the air, though which usually contains a small amount of CO_2, I have given the data on various animals taken by other investigators, who used atmospheric air instead of oxygen gas:

Subject	O_2-capacity		Observer	
Higher mammalia	18.C7 vols. %		HALDANE ('22)	
Eel	7 9	,,	JOLYET and REGNARD ('17)	
,,	14.5	,, (at 5°C)	KAWAMOTO	
,,	13.7	,, (at 17°C)	,,	
,,	12.2	,, (at 30°C)	,,	
Cauchna chilensis	6.1	.,	,,	('28)
Anadara inflata	5.1	.,	,,	(,,)

In this table given above we find that the blood of the eel studied by JOLYET and REGNARD gives a very small value of O_2-capacity compared with my own observation. The difference might be due to smaller value of relative volume of red corpuscles in the eel at the time of the examination by JOLYET and REGNARD but this supposition can not be tested from their data.

In regard to the values of the oxygen capacities it must be stated that the figures given in the Tables 6, 7, and 8 were conveniently transformed into the percentage saturation for the purpose of directly comparing with the values given by most other authors on the subject. It must also be stated that in my case the O_2-capacities were determined under oxygen pressure of 160 mm., while in most other experiments it was determined under the atmospheric pressure of 760 mm. Hg. and therefore it was necessary to reduce the maximum saturation in both cases into 100 per cent saturation, the rest of the values are to be calculated by taking the usual arithmetical proportion.

The values calculated by this method as just stated are given in Tables 9, 10, and 11 and in Fig. 4. As will be seen from the figure, the curves do not show clearly an *S* form within the range of my own observation, which is the usual form with the blood of higher mammalia as shown by BARCROFT ('14) and others, although the *S*-form may yet appear if the data are taken beyond the range where the oxygen tension is below 2 or 3 mm.. At any rate the resulting curve exhibits

TABLE 9.

(30±0.5°C).

O$_2$-tension X	O$_2$-% satur. Y	Calculated Y K=0.93	Difference
2.0 mm.	36.7	39.5	− 2.8
5.0	60.1	62.3	− 2.2
6.9	63.0	69.5	− 6.5
10.0	65.0	75.0	−10.0
20.0	83.2	86.9	− 3.7
30.0	90.5	91.7	− 1.2
50.0	95.5	92.6	+ 2.9
70.0	97.0	96.0	+ 1.0
100.0	98.0	97.2	+ 0.8
160.0	100.0	98.5	+ 1.5

TABLE 10.

(17±0.3°C).

O$_2$-tension X	O$_2$-% satur. Y	Calculated Y K=0.60	Difference
3.0	60.0	64.3	−4.3
5.6	71.5	77.0	−5.5
10.0	87.5	85.6	+1.9
20.0	95.5	92.6	+2.9
24.1	97.0	93.2	+3.8
30.0	97.2	94.8	+2.4
50.0	97.8	96.8	+1.0
70.0	97.9	97.6	+0.3
100.0	99.0	98.3	+0.7
160.0	100.0	99.0	+1.0

TABLE 11.

(5±0.3°C).

O$_2$-tension X	O$_2$-% satur. Y	Calculated Y K=1.0	Difference
3.0 mm.			
3.9	72.2	79.6	−7.4
5.0	83.0	83.4	−0.4
10.0	95.0	91.0	+4.0
15.0	96.0	93.9	+2.1
20.0	98.0	95.4	+2.6
30.0	98.5	96.9	+1.6
50.0	98.8	98.0	+0.8
70.0	99.0	98.9	+0.4
100.0	99.3	99.0	+0.3
160.0	100.0	99.5	+0.5
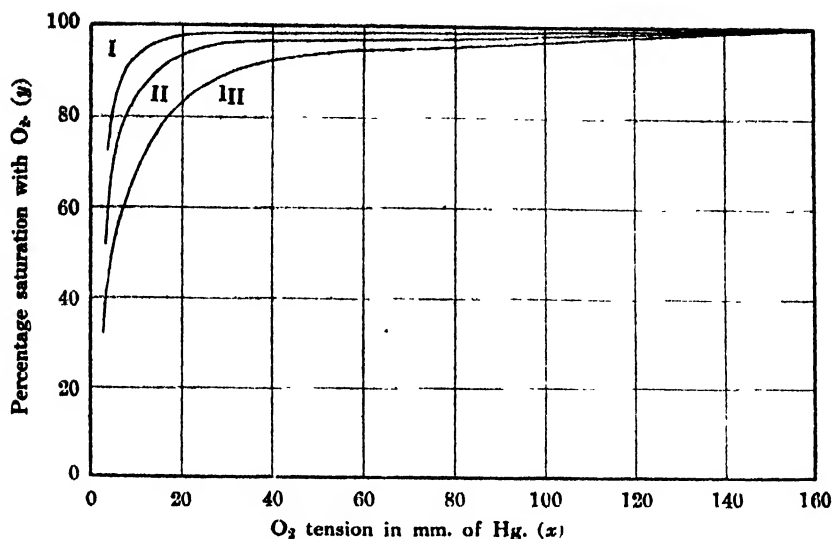
Fig. 4. Showing curves of haemoglobin dissociation at 5°C, 17°C and 30°C.
I....at 5°C, II....at 17 C, III... at 30°C.

a hyperbolic type and thus suggests naturally that the dissociation curve of the eel haemoglobin follows the law of monomolecule reaction.

I have tried to see whether or not the three curves (Fig. 4) would satisfy HILL's equation $\dfrac{y}{100} = \dfrac{Kx^{n}}{1 + Kx^{n}}$ putting the percentage of oxyhaemoglobin as y, and the oxygen pressure as x, and the percentage of the reduced haemoglobin as $(1-y)$. After trials, it was found that HILL's equation is satisfied only when the value of n, number of aggregation of haemoglobin molecules is made as small as unity (1) and the resulting values of K, the equilibrium constants, were found to be 1.0 at 5°C, 0.6 at 17°C and 0.33 at 30°C respectively.

Theoretically the values of n is unity only when the reaction takes place with single molecules of haemoglobin, and consequently we are forced to suppose that either the small value of n is characterristic of the blood of eel or that the reaction takes place with a very small aggregation of haemoglobin molecules.

The temperature coefficent, Q_{10}, was determined by the formula derived by SNYDER

$$Q_{10} = \left(\frac{k_{2}}{k_{1}}\right)^{\frac{10}{T_{2}-T_{1}}}$$

where k_1 and k_2 are the equilibrium constants at the absolute temperatures T_1 and T_2. The calculation was facilitated by taking $k = 100/K$, where K is equivalent to the K of HILL's equation as suggested by MACELA and SELISKAR ('25) and the following values were obtained.

$$\text{at } 5°C \qquad k = \frac{100}{1.00} = 100.0$$

$$\text{at } 17°C \qquad k = \frac{100}{0.6} = 166.6$$

$$\text{at } 30°C \qquad k = \frac{100}{0.33} = 303.0$$

The following values of Q_{10} were found, that is $Q_{10} = 1.53$ from 5° to 17°, $Q_{10} = 1.58$ from 17° to 30°C.

According to VAN'T HOFF (from KANITZ '15) the value of Q_{10} in the chemical reaction normally lies between 2 to 3. In the case of the equilibrium between haemoglobin and oxygen, it was found to be 2.5 for frog from 10° to 40°C, and 5.6 for man from 15°C to 35°C (MACELA and SELISKAR '25), while for the blood of eel its mean value was found to be 1.56 at 5° to 30°C. This small value of Q_{10} may be interpreted from the biological standpoint; that is the blood of the eel possesses greater affinity for oxygen compared with the blood of most other animals during the change of temperature. We notice then another peculiarity for eel blood in having the least value of Q_{10} compared with other animals so far experimented.

In order to compare the dissociation curves obtained by various authors the values of t_l and t_u were compared as was suggested by KROGH and LEITCH ('18). The value of t_u is especially convenient, since $1/K$ corresponds to the O_2 pressure where the Hb is just half saturated with oxygen. The values of t_l and t_u were directly read from Fig. 4, while the theoretical value of t_u was derived from $1/K$ in HILL's equation and the results are given below:

	at 5°C (mm. Hg.)	at 17°C (mm. Hg.)	at 30°C (mm. Hg.)
t_l observed	12.0	25.0	70.7
t_u ,,	1.5	2.0	4.0
t_u calculated	1.0	1.7	3.1

The observed values of t_u are compared with those obtained from other fishes.

Materials	t_i (mm. Hg.)	t_u (mm. Hg.)	temp. °C	Observer
Carp	10	2–8	17	KROGH & LEITCH ('19)
Eel	,,	,,	,,	,, ,, ,,
Pike	,,	,,	,,	,, ,, ,,
Trout	—	11	,,	,, ,, ,,
Eel	25	2	17	KAWAMOTO
Cod	70	18	15	KROGH & LEITCH ('19)
Plaice	40	10	,,	,, ,, ,,

From these values, KROGH and LEITCH conclude that the blood of fresh water fishes, excepting the trout, is better adapted to withstand against lower tension when compared with the blood of salt water fishes. Such an adaptation to the lower oxygen tension is vitally important for fresh water living fishes since in lakes and ponds, except in running water, the oxygen pressure is very variable and sinks often to very low values; while in sea water the oxygen pressure is practically constant and uniformly high. The form of the dissociation curve seems to suggest that the blood of the sea water fishes must be more sensitive to oxygen want which makes it more difficult to keep them in the aquarium than to keep fresh water fishes in captivity.

The chemical affinity of haemoglobin to oxygen can be measured from VAN'T HOFF's equation of the reaction isochore for the equiribrium between oxygen and haemoglobin:

$$\frac{d\ln K}{dT} = \frac{-q}{RT^2}$$

where K is the equilibrium constant, T the absolute temperature, R the gas constant, q the heat produced by the reaction of one gram-molecule of haemoglobin with oxygen. Assuming then that the value of q remains constant over the range of temperature in this experiment (5°—30°C), and integrating between limits T_1 and T_2, we find:

$$\ln K_2 - \ln K_1 = -\frac{q}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

where K_1 and K_2 are the equilibrium constants at the absolute

temperatures T_1 and T_2. If R be 1.985 and ordinary logarithms be employed, the equation for the heat produced is (NERNST '11)

$$q = \frac{5.571 \, (\log K_2 - \log K_1) \, T_1 T_2}{T_2 - T_1}$$

for the average value of temperatures T_1 and T_2.

Now, applying the equilibrium constants obtained from my experiments 1.0 at 5°, 0.6 at 17° and 0.33 at 30°C into the above formula, I found that the values of q was 51.200 calories at 11°C and 56.300 cal. at 23°C for the blood of eel, while MACELA and SELISKAR ('25) found in the blood of frog 13.290 cal. at 20°C and 26.850 cal. in human blood.

From these results, it becomes evident that the blood of the eel possesses greater affinity for oxygen than the blood of the frog and man, as was also found in connection with the oxygen capacity (MACELA & SELISKAR '25).

SUMMARY.

1. The hydrogen ion concentration of the blood of the eel was found to be constant during 43 days of starvation.
2. The standard value (median value) of the relative volume of red corpuscles was determined in this experiment as 39 per cent.
3. The oxygen capacity of the blood is directly proportional to the relative volume of red corpuscles in the limit of 23 to 53 per cent.
4. The haemoglobin dissociation curves are satisfactorily represented by HILL's equation by putting the value of $n=1$ within the limit of my observation.
5. The mean value of Q_{10} is 1.56 at 5° to 30°C and such small value may indicate the stability for oxygen affinity at the change of temperature.
6. Greater amount of heat produced during chemical reaction between oxygen and haemoglobin may be taken to mean that the blood of the eel possesses greater affinity for oxygen than that of the frog and human blood.

The writer wishes to express his indebtness to Prof. Dr. S. HATAI and Dr. S. KOMINE for very valuable suggestions and advices.

BIBLIOGRAPHY.

1) BARCROFT, J. 1914. The Respiratory Function of the Blood. London. pp. 36, 63–65.
2) DOMARUS, A. V. 1921. Methodik der Blutuntersuchung. Berlin S. 13.
3) HALDANE, J. S. 1922. Respiration. London. p. 62.
4) HALL, F. G., I. E. GRAY and S. LEPKOVSKY. 1926. J. Biol. Chem., LXVII. p. 549.
5) HILL, A. V. 1910. J. Physiol., XL, pp. iv-vii.
6) JOLYET, F. et P. REGNARD. 1877. Arch. de Physiol. norm. et pathol., (2) 5, 684.
7) KANITZ, A. 1915. Temperature und Lebensvorgänge.
8) KAWAMOTO, N. 1928. Sci. Rep., Tôhoku Imp. Univ., 4th Ser., Biology, III. No. 4, Fasc. 1, pp 561-575.
9) KAWAMOTO, N. 1929. Sci. Rep., Tôhoku Imp. Univ., 4th Ser., Biology, IV. No. 4, pp. 635–641.
10) KROGH, A. and L. LEITCH. 1919. J. Physiol., LII, pp. 288-300.
11) MACELA, I. and A. SELISKAR. 1925. J. Physiol., LX. 5.
12) MARUKAWA, H. 1916. J. Imp. Fish. Inst., XI, 4, pp. 14.
13) NERNST, W. 1911. Theoretical Chemistry. London, p. 660.
14) SCHAEFER, A. A. 1925. J. Gen. Physiol., VIII. 3. p. 341.
15) VAN SLYKE, D. D. and J. M. NEIL. 1924. J. Biol. Chem., LXI, pp. 523-573.
16) VAN SLYKE, D. D. 1927. J. Biol. Chem., LXII, 1, pp. 121-126.
17) WASTL, H, 1928. Biochem. Zeitsch., CLXXXXVII, 4-6. S. 363-380.
18) OGURA, K. 1920. The Method of Statistical Investigation. Ōsaka. p. 68.

Meiosis im Oogonium von *Sargassum Horneri* (Turn.) Ag.

Von

Sakuichi Okabe.

(Biologisches Institut der Kaiserlichen Tôhoku Universität, Sendai.)

Unter den Fucaceen wurde die Gattung *Fucus* früher schon von Strasburger (1897), Farmer und Williams (1898) und Yamanouchi (1909) cytologisch untersucht; besonders der letztgenannte Autor hat eine sehr schöne Arbeit über die Reduktionsteilung und Befruchtung bei *Fucus vesciculosis* geschrieben.

Über die Karyokinese von *Sargassum* publizierten Tahara und Shimotomai (1926) eine kurze Mitteilung, in der sie hauptsächlich die erste Metaphase der Reduktionsteilung des Oogoniumkerns von *Sargassum enerve* behandelten; sie konnten 32 Gemini und je ein Centrosom an jedem Pol erkennen. Im vorigen Jahre hat Kunieda über die Kernteilung und Befruchtung von *Sargassum Horneri* etwas umfangreichere Ergebnisse mitgeteilt. Aber seine Resultate stimmen mit denen der vorhergehenden Forscher bezüglich der Chromosomenzahl und des Befruchtungsvorgangs nicht überein. Shimotomai (1928) hat bei *Cystophyllum sisymbrioides* ein ganz gleiches Resultat wie bei *Sargassum enerve* erhalten. Alle eben genannte Autoren haben aber die Einzelheiten der Kernteilungsvorgänge nicht ausführlich beschrieben.

In diesem Frühling veranlasste mich Herr Prof. M. Tahara, die Vorgänge der Reduktionsteilung von *Sargassum* noch näher zu untersuchen. Deshalb habe ich bei meinem Aufenthalt auf der biologischen Station der kaiserlichen Tokyo-Universität in Misaki die Karyokinese im Oogonium von *Sargassum Horneri* beobachtet. Gleichzeitig hat Herr Prof. M. Tahara auf der biologischen Station der hiesigen Universität in Asamushi die Oogenese von *Coccophora Langsdorfii*, einer im Japanischen Meer einheimischen Art, ausführlich untersucht; seine Resultate wurden schon in der vorhergehenden Nummer dieser Zeitschrift veröffentlicht.

Im Frühjahr wuchern in Misaki verschiedene Arten von *Sargassum*.

Ich habe aber ausschliesslich *Sargassum Horneri* als günstiges Material für meine Untersuchung benutzt, denn der Oogoniumkern dieser Alge ist grösser als der der übrigen, und überdies ist die Umgebung des Oogoniumkerns bei jener Art gewöhnlich chromatophorenarm. Die Karyokinese des Oogoniumkerns beginnt im Konzeptakel einige Stunden vor der Oogonienentleerung. Da die Entleerung der Oogonien, wie TAHARA (1913) schon näher untersucht hat, simultan und periodisch an einem bestimmten Tage vor sich geht, so kann man häufig um diese Zeit verschiedene karyokinetische Phasen beobachten. Wenn der Oogoniumkern in die meiotische Phase eintritt, so erfolgt auch eine merkwürdige Veränderung im Cytoplasma, d. h. die bis dahin in der Peripherie des Oogoniums gelegenen Chromatophoren wandern allmählich in die Umgebung des Kerns (Textfig. 1). Dieser Zustand lässt sich selbst beim lebenden Material mit schwacher Vergrösserung ohne Schwierigkeit klar erkennen. Darum konnte ich genug Material in den geeigneten Phasen der meiotischen Kernteilung von *Sargassum Horneri* fixieren.

Bei der karyologischen Erforschung der Algen muss man besonders auf die Fixierung des Materials achtgeben. TAHARA hat auf Grund langer Erfahrungen eine Flüssigkeit gefunden, die sich zur Fixierung des Oogoniumkerns von *Sargassum* und nahe verwandten Gattungen besonders eignet. Sie besteht aus: —

Stocklösung von Chromsäure (Seewasser 98 ccm, gesättigte
    Lösung von Chromsäure 2 ccm)...............65 ccm
Seewasser . . . .35 ccm
2% Osmiumsäure...........................5 ccm
Eisessigsäure .... 2.5 ccm

Diese Flüssigkeit ähnelt der FLEMMINGlösung in der Bonner Konzentration bez. des Verhältnisses der Osmiumsäure und Essigsäure, aber die der Chromsäure ist sehr viel grösser. Bei der Benutzung dieser Lösung darf man aber die Konzentration der Chromsäure je nach dem Material etwas verändern. Das vorliegende Material wurde mit dieser Flüssigkeit meistens 4–7 Stunden lang fixiert, noch längeres Liegenlassen in dieser Lösung ist nach meiner Erfahrung in diesem Falle ganz unnötig. Wenn man mit diesem Fixierungsmittel gute Resultate gewinnen will, so ist es wesentlich, die Rezeptakel so klein wie möglich, etwa 1 mm dick, quer zur Längsachse, zu zerschneiden.

Das fixierte Material wird nach vorsichtiger Behandlung mit verschieden prozentigem Alkohol und Chloroform in Paraffin von 48°C Schmelzpunkt eingebettet. Das Einbetten des Oogoniums machte meist nicht so grosse Schwierigkeiten, wie ich zunächst befürchtet hatte. Die Färbung der 5-15 μ dick geschnittenen Paraffinschnitte erfolgte mit Safranin-Lichtgrün und HEIDENHAINs Eisenalaunhämatoxylin.


a   b
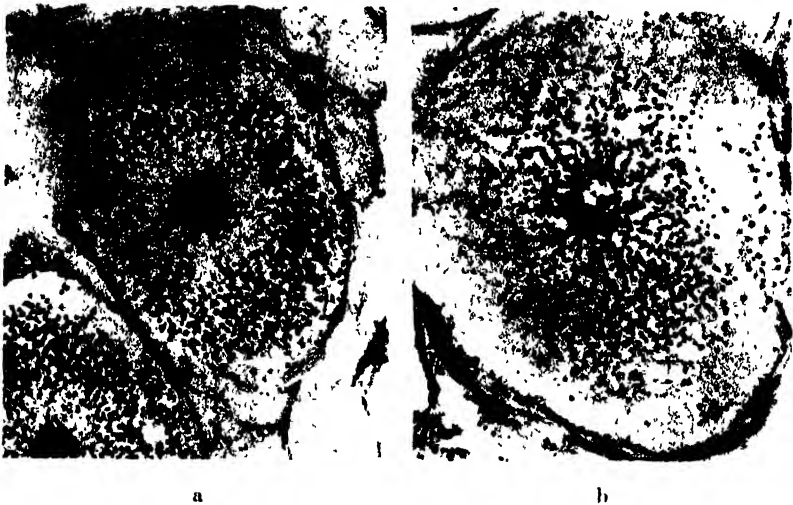
Textfig. 1. Oogonien von *Sargassum Horneri.* ca. ×200.

a: Ruhestadium. b: Synapsisstadium. Die Chromatophoren sind im Cytoplasma gleichmässig zerstreut.

Im vollständigen Ruhestadium enthält der Oogoniumkern gewöhnlich einen grossen Nukleolus und ein kleines, stark tingierbares Stäbchen, welches zuweilen nadel- oder ringförmig ist, und viele kleine Chromatinkörner, die an den in der Kernhöhle in verschiedener Richtung durchlaufenden Lininfäden verteilt sind (Pl. XXVII, Fig. 1). In diesem Stadium ist es leicht, im Cytoplasma des Oogoniums drei Schichten zu unterscheiden (Textfig. 1. a). Die innerste Schicht ist dünn und umgibt direkt den Kern als eine dichte Plasmascheide, die am fixierten Material sehr feine, wabige Struktur zeigt. In dieser Plasmascheide konnte ich noch in diesem Stadium weder Strahlung noch ein Centrosom beobachten. Die nächste äusere Schicht ist bei weitem breiter als die erste und sieht durchsichtig aus, weil die Wabenstruktur bei dieser

Schicht viel lockerer ist. Die äuserste Schicht mit einer grossen
Menge von Chromatophoren ist wieder ganz undurchsichtig. Wenn
der Kern aus seiner Ruhe aufgeweckt wird, so beginnen die bis dahin
auf die äuserste Schicht beschränkten Chromatophoren sich gleichmässig
über das ganze Cytoplasma zu zerstreuen (Textfig. 1. b). Dabei ist
es aber sehr merkwürdig, dass ein kleiner, an die Kernmembran
angrenzender Bezirk ganz frei von Chromatophoren zu bleiben pflegt.
Diesen Bezirk möchte ich vorläufig Centrosom-Bezirk nennen, weil in
ihm das Centrosom später auftritt. Der Centrosom-Bezirk dürfte wohl
durch Anhäufung der innersten Plasmaschicht entstanden sein. Das
synaptische Knäuel bildet sich in der Kerngegend, welche direkt an
diesen Bezirk angrenzt.

a   b
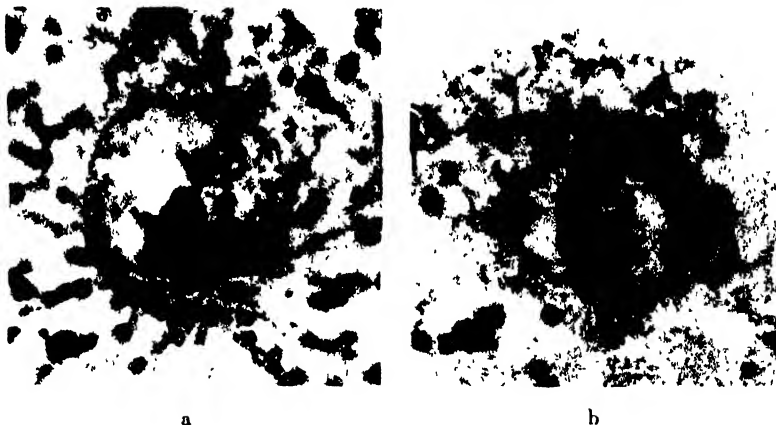
Textfig. 2. Zwei Stadien heterotyper Teilung des Oogoniumkerns
von *Sargassum Horneri.*

a: Synapsisstadium. ca. ×850. b: frühere Metaphase, wo der Nukleolus
sehr dünn wird und die Kernmembran noch teilweise intakt bleibt. ca. ×1120.

Pl. XXVII, Fig. 2 stellt ein früheres Synapsisstadium dar, wo ein
kleines, synaptisches Knäuel gedrängt an einer Seite des Kerns vorragt.
Zu dieser Zeit wird der Centrosom-Bezirk sehr plasmareich und zeigt
deutliche Strahlungsstruktur, während die dichte Plasmascheide, die
vorher als innerste Plasmaschicht die ganze Umgebung des Kerns
gleichmässig umschlossen hatte, allmählich dünner und dünner wird.
In der Kernhöhle finden wir eine zuweilen stark tingierbare Substanz,

welche nicht als eine Art Chromatin betrachtet werden kann und je nach dem Modus der Fixierung ganz verschieden gestaltet ist (Pl. XXVII, Fig. 2–6 und Pl. XXVIII, Fig. 7–10). Mit dem Fortschreiten des Stadiums erstrecken sich die Fäden des synaptischen Knäuels in die Kernhöhle, wobei die Strahlung in dem Centrosom-Bezirk deutlicher wird (Pl. XXVII, Fig. 3, Textfig. 1. b und Textfig. 2. a). In der Mitte dieser Strahlung suchte ich mit grosser Geduld nach dem Centrosom, aber stets vergeblich. Im nächsten Stadium tritt eine grosse Veränderung nicht nur in der Kernhöhle, sondern auch in der äusseren Plasmamasse ein : die synaptischen Elemente zerstreuen sich, ohne einen kontinuierlichen Spiremfaden zu bilden, als einzelne Segmente in die Kernhöhle, und der Centrosom-Bezirk wird auch in diesem Stadium in zwei geteilt (Pl. XXVII, Fig. 4). YAMANOUCHI (1909) hat schon bei *Fucus* eine ähnliche Phase beobachtet. Er schreibt : „The two arms of each of these loops then separate at the bend — the point of connection — and form a pair of bivalent chromosomes in prophase of the first division. The bivalent chromosomes remain for a while at spot where they were grouped in synapsis, and then become distributed in the nuclear cavity (fig. 4) ". Nach TAHARAs Untersuchung erscheint ein kontinuierlicher Spiremfaden, wie bei höheren Pflanzen, in dem entsprechenden Stadium von *Coccophora Langsdorfii*. Obwohl YAMANOUCHI bei *Fucus* Telosynapsis nachgewiesen hat, vermag ich doch nicht zu behaupten, dass sie auch bei meinem Material vorkommt.

Erst zu dieser Zeit kann man je ein Centrosom in der Mitte jeder Strahlung mit Bestimmheit konstatieren. Es is auffallend, dass in der Nähe des Nukleolus fast stets ein stark tingierbares Körperchen vorhanden ist. Etwas Ähnliches wurde von WILLIAMS (1904) bei *Dictyota* und von CARTER (1927) bei *Padina* bemerkt und als „ chromophilous spherule " bezeichnet. TAHARA hat das auch bei *Coccophora* gefunden. Im darauf folgenden Stadium werden die in der Kernhöhle zerstreuten Bivalentchromosomen kürzer und dicker, und dann folgt das Diakinesestadium (Pl. XXVII, Fig. 5 und 6, und Pl. XXVIII, Fig. 7). Die beiden Strahlungen mit je einem Centrosom in der Mitte ziehen sich längs der Kernmembran auseinander, bis sie sich etwa um 130° voneinander entfernen (Pl. XXVIII, Fig. 7). Pl. XXIX, Fig. 8 zeigt ein späteres Diakinesestadium, in dem ein Nukleolus, 32 Gemini und zwei Spinndelanlagen zu sehen sind. Die Bildung der Spindelfasern beginnt

an der Stelle, wo schon Strahlungen vorhanden sind. Die erst noch
kurzen Fasern wachsen dann allmählich und dringen in die Kernhöhle
ein. Da das Auftreten der Spindelanlagen gewöhnlich nicht gleichzeitig
ist, wird zuweilen ein Spindelfaserbüschel viel grösser als das andere.
Mit dem Wachsen der internuklearen Spindelfasern werden die
Strahlungen im Centrosom-Bezirk sehr undeutlich. Die „chromophilous
spherule" ist in diesem Stadium schon verschwunden. Wenn eine
bipolige Spindel, die sich stets von der Achse des Kerns etwas entfernt
bildet, fertig ist, ziehen sich die 32 Gemini regellos in dem mittleren
Teil der Spindel zusammen. Die Kernmembran und der Nukleolus
bleiben noch intakt. Zu dieser Zeit wird die Strahlung wieder auf
einem Pol deutlich (Pl. XXVIII, Fig. 9). Nach TAHARA verläuft dasselbe
Stadium bei *Coccophora Langsdorfii* etwas verschieden vom meinigen;
bei jener Pflanze verschwindet nämlich der Nukleolus schon im
früheren Diakinesestadium, und an keinem Pol der Spindel ist ein
Centrosom nachweisbar. Pl. XXVIII, Fig. 10 gibt dasselbe Stadium
bei meiner Pflanze in Polansicht wieder, wobei sich die 32 Chromo-
somen ohne Schwierigkeit zählen lassen.

Hier werde ich das Centrosom kurz beschreiben. Obwohl KUNIEDA
bei dieser Alge kein Centrosom bemerkt hat, konnte ich es wie bei
Fucus, Cystophyllum und *Sargassum enerve* in verschiedenen karyo-
kinetischen Stadien, ausgenommen bei der Synapsis und Interkinese,
beobachten. In Seitenansicht erscheint das Centrosom meist punkt-
oder hantelförmig, aber in Polansicht ist es in zwei Körnchen geteilt
(Pl. XXVIII, Fig. 11). Durch veränderte Einstellung des Mikroskops ist
dieser Zustand an beiden Polen leicht zu erkennen. Das hantelförmige
Aussehen, das von SWINGLE (1897) bei *Stypocaulon*, von STRASBURGER
(1897) bei *Fucus*, von WILLIAMS (1904) bei *Dictyota* und von CARTER
(1927) bei *Padina* nachgewiesen wurde, dürfte auf dem engen Zusam-
menliegen beider Körnchen beruhen.

Vor der Auflösung der Kernmembran und des Nukleolus ordnen sich
die Gemini auf der Äquatorialebene (Pl. XXVIII, Fig. 12 und Textfig.
2, b). Die vollständige Metaphase erfolgt aber erst nach Auflösung
der Kernmembran, die gewöhnlich von einer Seite nach der anderen
fortschreitet (Pl. XXIX, Fig. 13 und 14). Wie Pl. XXIX, Fig. 14
klar zeigt, sind so grosse Unterschiede unter den Chromosomen nicht
wahrnehmbar, ausgenommen dass nur ein Geminus etwas grösser als

die übrigen ist. KUNIEDA hat schon bei dieser Alge 16 haploide und
32 diploide Chromosomen gezählt. Er gewann sein Resultat aus der
Beobachtung der Oogenese, Spermatogenese und der somatischen
Teilungen bei der Keimentwicklung. Wodurch diese Nichtüberein-
stimmung mit meinem Ergebnis entstanden ist, lässt sich im Augenblick
nicht erklären. Bei nächster Gelegenheit möchte ich auch die Sper-
matogenese und Keimentwicklung cytologisch untersuchen. Nach
neueren Untersuchungen verschiedener Forscher besitzen unter den
Fucaceen manche Arten, nämlich *Fucus vesciculosus* (YAMANOUCHI
1909), *Sargassum enerve* (TAHARA und SHIMOTOMAI 1926), *Cystophyllum
sisymbrioides* (SHIMOTOMAI 1928) und *Coccophora Langsdorfii* (TAHARA
1929) 32 reduzierte chromosomen. Es ist sehr wünschenswert, die
Chromosomenzahlen der gesamten Arten verschiedener Gattungen von
Fucaceen genau zu untersuchen.

Die Anaphase geht regelmässig vor sich. Die reduzierten Chro-
mosomen wandern nach jedem Pol, wo das in zwei geteilte Centrosom
sehr deutlich zu sehen ist (Pl. XXIX, Fig. 15), In der Telophase ist
der Zwischenraum beider Tochterkernanlagen sehr ausgedehnt. Die an
einer Seite des Kerns gedrückten Chromosomen lösen sich allmählich
auf, und zugleich tritt wieder eine dichte Plasmamasse direkt ausser-
halb des Kerns, an der poligen Seite beschränkt, auf (Pl. XXIX, Fig.
16). Danach vergrössert sich der Kern allmählich und zeigt ein
feines Netzwerk in seinem Innern (Pl. XXIX, Fig. 17).

Ohne lange Pause beginnt homöotype Teilung. In der Prophase
treten wieder 32 univalente Chromosomen auf. In der späteren
Prophase treten zwei Centrosomen in der Plasmamasse auf, aber
merkwürdigerweise fehlen die Strahlungen um sie herum (Pl. XXIX,
Fig. 19). Die Spindelbildung verläuft in gleicher Weise wie die
heterotype Teilung (Pl. XXIX, Fig. 20). Pl. XXIX, Fig. 21 zeigt eine
spätere Prophase, in der die Chromosomen sich an der Stelle sammeln,
wo die beiden Spindelfaserbüschel zusammentreffen. Die schon ge-
spaltenen, hantelförmigen Chromosomen ordnen sich regelmässig in der
Äquatorialplatte, und die Kernmembran und der Nukleolus verschwinden
kurz danach (Pl. XXIX, Fig. 22 und 23). Die Anaphase und Telophase
verlaufen regelmässig ebenso wie die vorhergehende Teilung, und vier
Kerne werden daraus gebildet.

Die dritte Teilung, welche acht Kerne in einem Oogonium liefert,
verläuft ganz gleich wie die homöotype.

ZUSAMMENFASSUNG.

1.　Drei aufeinander folgende Kernteilungen treten in einem Oogonium von *Sargassum Horneri* auf, von denen die ersten zwei allotyp sind.

2.　In der Prophase der heterotypen Teilung gibt es die typische Synapsis und Diakinese, aber nicht das Spiremstadium.

3.　Die Kernmembran und der Nukleolus verschwinden, wenn die Kernplatte da ist.

4.　Bei der Karyokinese sind einige Differenzierungen im Cytoplasma sehr deutlich, nämlich die Strahlung und das Centrosom.

5.　Diese Pflanze zeigt 32 Haploidchromosomen.　Also stimmt mein Resultat mit dem KUNIEDAS nicht überein.

Zum Schluss sei es mir gestattet, meinem hochverehrten Lehrer, Herrn Prof. Dr. M. TAHARA, für seine Anregung und seine vielseitigen Ratschläge meinen besten Dank auszusprechen.　Ich schulde auch Herrn Prof. Dr. N. YATSU, Direktor der Biologischen Station zu Misaki, für seine freundliche Unterstützuug herzlichen Dank.

den 11. Oktober 1929.

LITERATURVERZEICIINIS.

1) CARTER, P. W. 1927. The life-history of *Padina pavonia*. 1. The structure and cytology of the tetrasporangial plant. Ann. of Bot., Vol. XLI, S. 139–159.

2) FARMER, J. B. and WILLIAMS, J. Ll. 1898. Contributions to our knowledge of the *Fucaceae*: their life-history and cytology. Phil. Trans. Roy. Soc. London, B., Vol. 190, S. 623–645.

3) KUNIEDA, II. 1928. On the development of the sexual organs and embryogeny in *Sargassum Horneri*, Ag. Jour. of Coll. of Agriculture, Imp. Univ. Tokyo, Vol. IX, S. 383–396.

4) SHIMOTOMAI, N. 1928. Karyokinese im Oogonium von *Cystophyllum sisymbrioides*, J. Ag. Science Report, Tôhoku Imp. Univ., 4th Ser., Vol. III, S. 577–579.

5) STRASBURGER, E. 1897. Kernteilung und Befruchtung bei *Fucus*. Jahrb. f. wiss. Bot., Bd. 30, S. 351–374.

6) SWINGLE, W. T. 1897. Zur Kenntniss der Kern- und Zellteilung bei den Sphacelariaceen. Jahrb. f. wiss. Bot., Bd, 30, S. 297–350.

7) TAHARA, M. und SHIMOTOMAI, N. 1926. Mitosen bei *Sargassum*. Science Rep 4th Ser., Vol. I, S. 189–192.

8) TAHARA, M. 1929. Ovogenesis in *Coccophora Langsdorfii* (TURN.) GREV. Science Rep. 4th Ser., Vol. IV, S. 551-556.

9) WILLIAMS, J. Ll. 1904. Studies in *Dictyotaceae*. 1. The cytology of the tetrasporangium and the germinating tetraspore. Ann. of Bot., Vol. XVIII, S. 141-158.

10) YAMANOUCHI, S. 1909. Mitosis in *Fucus*. Bot. Gaz., Vol. XLVII, S. 173-196.

ERKLÄRUNG DER TAFELN.

Sämtliche Bilder wurden mit Hilfe eines ABBEschen Zeichenapparats gezeichnet, unter Benutzung des LEITZschen Achromat-Objektivs $\frac{1}{12}$ und des ZEISSschen Okulars ×15. Vergrösserung 1950.

TAFEL XXVII.

Fig. 1. Kern in Ruhe.

Fig. 2-3. Synapsis.

Fig. 4. Späteres Stadium, wo die Segmente des synaptischen Knäuels in der Kernhöhle zerstreut liegen.

Fig. 5-6. Noch späteres Stadium in zwei optischen Schnitten.

TAFEL XXVIII.

Fig. 7. Frühere Diakinese.

Fig. 8. Spätere Diakinese. Die Spindelbildung beginnt.

Fig. 9. Noch späteres Stadium in Seitenansicht.

Fig. 10. Dasselbe Stadium in Polansicht.

Fig. 11. Polansicht der Spindel. Zwei winzige Körnchen, d. h. Centriolen, sind sichtbar.

Fig. 12. Frühere Metaphase der heterotypen Teilung.

TAFEL XXIX.

Fig. 13. Heterotype Metaphase in Seitenansicht.

Fig. 14. Dasselbe Stadium in Polansicht, wobei 32 Chromosomen deutlich zu sehen sind.

Fig. 15. Anaphase.

Fig. 16. Telophase.

Fig. 17. Interkinese.

Fig. 18-21. Prophasen der homöotypen Teilung.

Fig. 22. Frühere Metaphase der homöotypen Teilung.

Fig. 23. Homöotype Metaphase.

Fig. 24. Homöotype Anaphase.
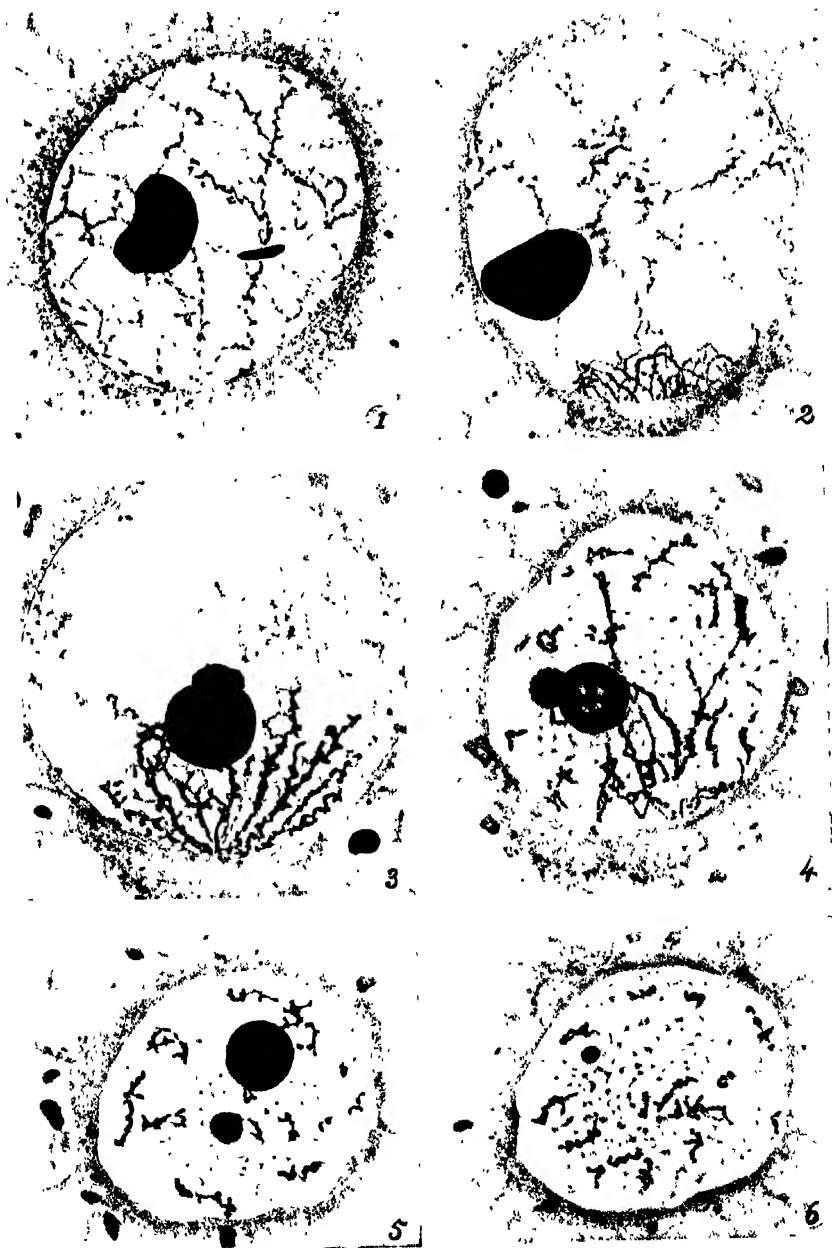
S. OKABE: Meiosis im Oogonium von *Sargassum Horneri*.
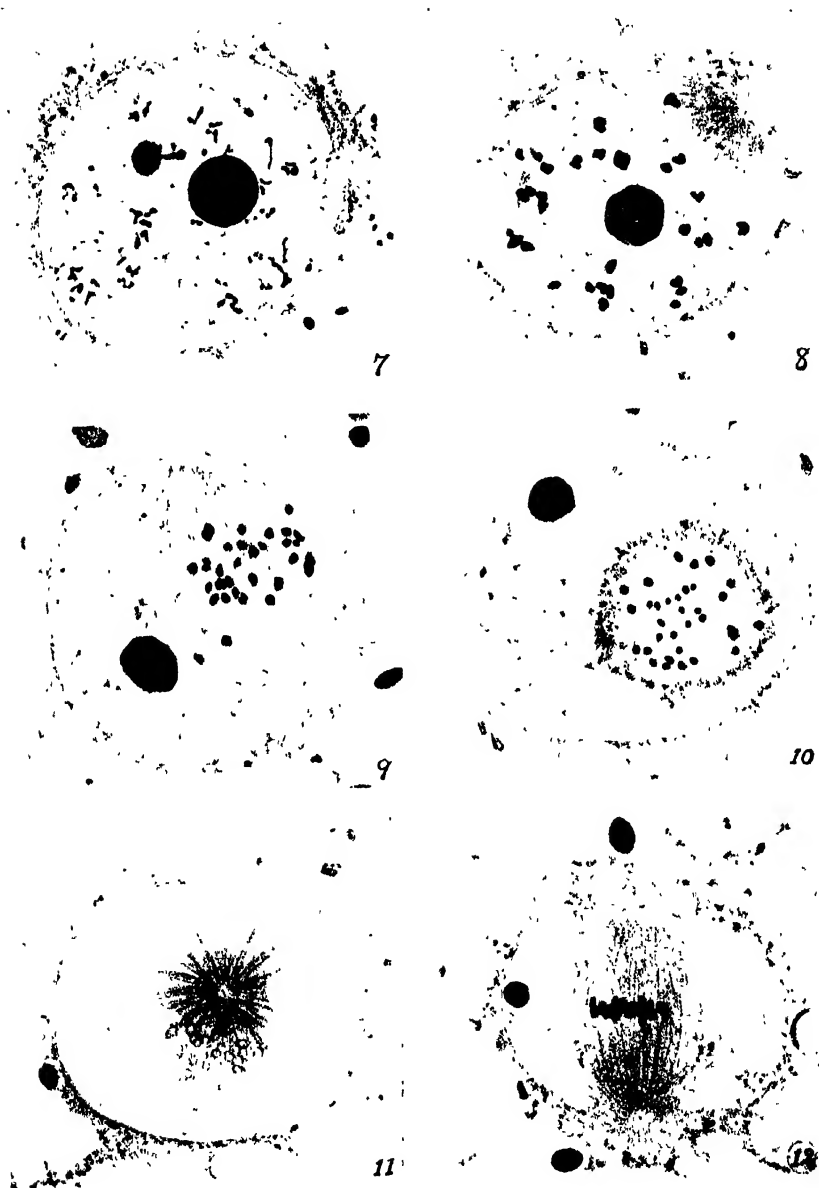
S. Okabe: Meiosis im Oogonium von *Sargassum Horneri*.
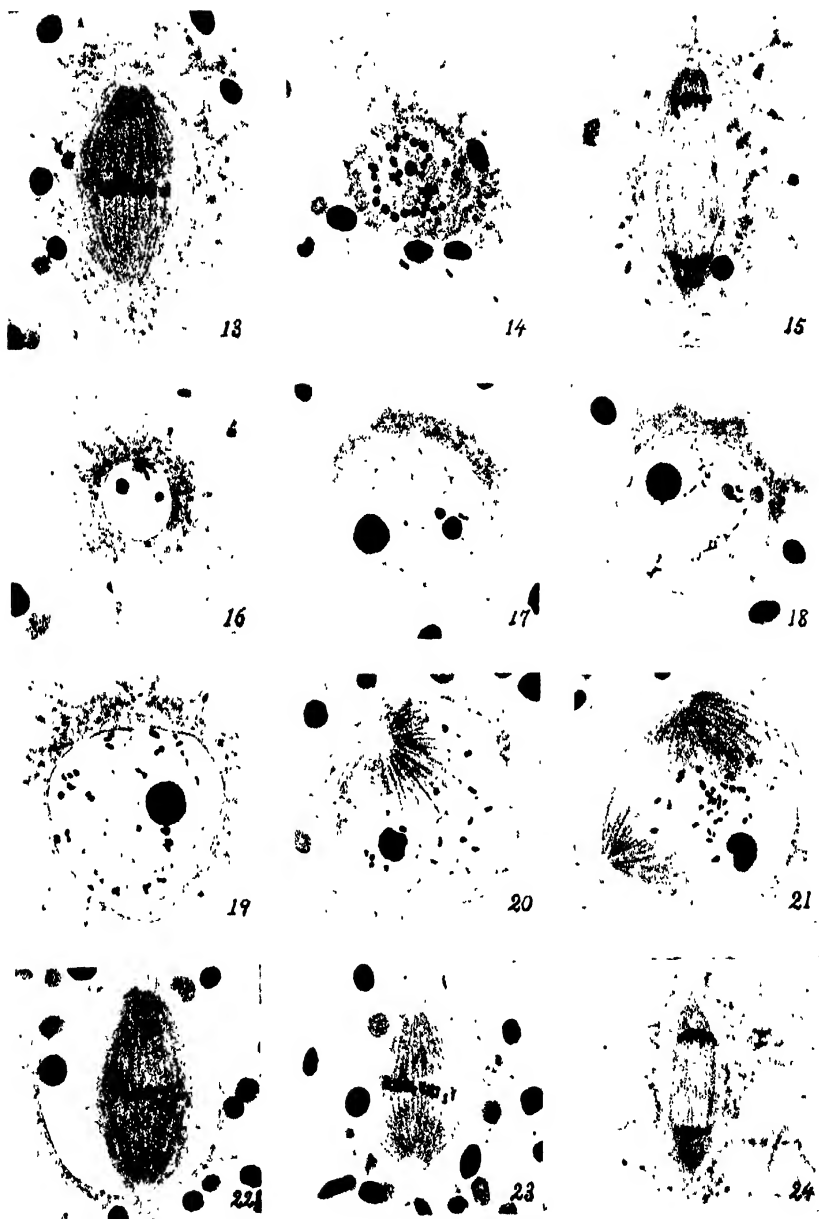
O. OKABE: Meiosis im Oogonium von *Sargassum Horneri*.

Report of the Biological Survey of Mutsu Bay.
14. *Melitodes mutsu*, a new Gorgonid-Coral.[1]

By

HIROMU MINOBE.

(Zoological Institute, Faculty of Agriculture, Tôkyo Imperial University.)

(With two Text-figures.)

Through the courtesy of Professor HÔZAWA I had occasion to examine a colony and a few fragments of a Gorgonid-coral from Mutsu Bay. They were secured by Professor TAHARA on the coast of Bentenjima, Ômasaki, as well as by Professor HZOAWA near Sai, Takaisozaki. On examination the specimens before me proved to be of a species of the Genus Melitodes hitherto unrecorded. I propose to call it *Melitodes mutsu.*
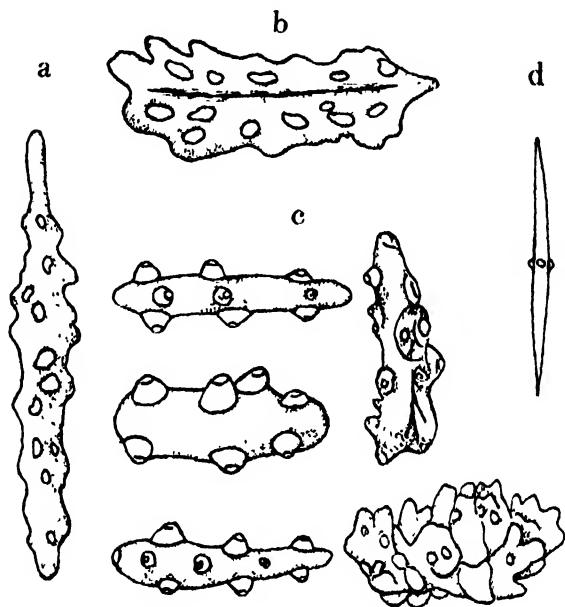
Melitodes mutsu, n. sp.

In the type-specimen which was obtained at Bentenjima, the colony is of a brick red colour and measures 6.5 cm. high and 9 cm. across in the terminal portion (Text-fig. 1). It consists of some short basal stems, each of which is 7.5 mm. in diameter and almost immediately breaks up into branches dichotomously divided and flattened especially in the distal part. The branches and branchlets are arranged in almost parallel planes and present anastomoses at irregular intervals. Distributed in two rows are polypes which are

Text-fig. 1. *Melitodes mutsu,* n. sp. (× ⅔)

1 mm. high by 1.5 mm. wide. The spicules of calyx walls are quite similar to those found in *Melitodes densa* KÜKENTHAL. The polype spicules (Text-fig. 2, a) are warty spindles, about 0.2 mm. long and arranged in transverse rows, while the tentacular spicules (Text-fig. 2,

Text-fig. 2. Spicules of *Melitodes mutsu*, n. sp.
a, Polype spicule.   b, Tentacular spicule.   c, Spicules of coenenchyme.
d, Spicule of node. (a, c, d×300; b×350)

b) are very thick warty spindles with a median keel on the outer side and disposed in eight distally converged tentacular groups. There are fairly numerous spicules in the coenenchyme, which vary considerably in form but not in size (Text-fig. 2, c). They are thick warty spindles and measure 0.1—0.15 mm. in length. The above-mentioned three types of spicules are of a yellowish vermilion colour. The spicules found in the node are slender and spindle-shaped with both ends sharply pointed and are provided with minute projections at the middle (Text-fig. 2, d).

Localities. — Bentenjima, Ômasaki ; Takaisozaki, Sai.

Remarks. — *Melitodes mutus*, n. sp. is very closely allied to *Melitodes*

densa KÜKENTHAL. The characteristic by which the former may be distinguished from the latter exists in the features of the spicules contained in the nodes.

LITERATURE CITED.

1) KÜKENTHAL, W. Melitodidae: Beitrage zur Naturgeschichte Ostasiens, Japanische Gorgoniden Teil II.

2) ——. Die Gorgonidenfamilie der Melitodidae Verr·; Zool. Anzeiger Bd. XXXIII.

3) ——. System und Stammesgeschichte der Melitodae: Zool. Anzeiger Bd. XLVII.

4) ——. Melitodidae: Wissenschaft. Ergebniss der Deutschen Tiefseeexpedition Val-divia Bd. XXIII 2^1 and 2^2.